# AUTOMATIC METHODS OF ANALYSIS

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# Preface

Miniaturization and the reduction of human intervention are two clear trends in the technological developments which characterize the last years of this century. Analytical chemistry has not ignored these trends, as has been reflected in most of the innovations introduced in this discipline for some time now.

For a variety of reasons, the automation of laboratory processes is an aspect of growing theoretical and practical interest. This was one of the reasons for us to write a monograph on the subject with the aim of presenting a rational overview of the areas most strongly influenced by the advances in automation. It was not our aim to present an exhaustive review of the large variety of alternatives reported and applied in the field of automatic methods of analysis, which would have been the subject of an encyclopaedia rather than a single volume.

The different topics dealt with were chosen according to various criteria such as the degree of consolidation, scope of application and most promising trends. The monograph consists of four parts. The first, after dealing with the basic principles behind the automation of laboratory processes (Chapter 1) and the role of computers in this context (Chapter 2), describes automatic systems for sampling (Chapter 3) and sample treatment (Chapter 4). The second part discusses the principles and commonest components of the principal types of analysers, namely continuous (Chapters 5-7), batch (Chapter 8) and robotic (Chapter 9). The third part is devoted to the automation of analytical instrumentation: spectroscopic (Chapter 10), electroanalytical (Chapter 11) and chromatographic (Chapter 12) techniques, and titrators (Chapter 13). The last part presents some examples of the application of automation to three of the most representative areas of chemical analysis: clinical chemistry (Chapter 14), environmental pollution monitoring (Chapter 15) and industrial process control (Chapter 16).

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THE AUTHORS

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# 1

# Fundamentals of laboratory automation

## 1.1 INTRODUCTION

The partial or complete replacement of human participation in laboratory processes is a growing trend that started in the 1960s and consolidated in the next decade [1-3]. So much so that, in the course of time, the distinction between modern and classical analytical chemistry will predictably be closely related to that between automated and non-automated analytical procedures.

This trend is the result of a variety of causes. On the one hand, automation relies on the spectacular advances in micromechanics, microelectronics and microcomputer science [4,5]. Microcomputers, whether or not used as microprocessors, are by now as commonplace as balances in laboratories, whether devoted to routine control or research and development. On the other hand, society's needs, frequently turned into demands, have grown to the extent of posing problems unimaginable a few years ago [6]. Such demands are not only the need for greater and more rapidly obtained analytical information, but also the laboratory's need to respond to new, pressing questions. All areas of social interest (health, ecology, industry, nutrition) are profoundly affected by the need for increasingly stricter control of a growing number of samples in which a large number of analytes are to be determined at increasingly low concentrations. Developing new products and solving problems previously not encountered are other aspects strongly dependent on laboratory work.

Cost reduction is yet another aim of the growing trend towards automation. The progressive elimination of human participation in laboratory processes or stages, wherever feasible and sensible, improves economic yields and reduces the cost of performance. Surveys carried out by many private and public US laboratories show the need for automation. It is interesting that most of such studies also show that automation does not necessarily result in redundancy as the growing number of problems encountered require new sections or even specialized laboratories to be started by the same organization. This results in retraining or even in the engagement of further employees. Obviously, automation affects unskilled workers to a greater extent on account of their more difficult retraining. Unmanned laboratories are as yet an utopia: there

is a series of tasks, both intellectual and manual, still reserved for humans. Although the number will probably decrease in time, there will always be a limit to complete automation, a limit that will probably have been established by the end of the century.

Automation, as shown later, offers undeniable advantages. Thus, it is hardly surprising that most analytical instrumentation manufacturers are tending to increase the degree of automation of their commercial ranges; the trend of laboratory managers to invest increasing funds in these automated instruments is also not surprising. However, as with any technological innovation, automation frequently meets with reluctant attitudes from those who think that adherence to a given change will invariably give rise to a variety of complications —a reactionary attitude in this respect can only result in more serious problems in the future. The complete opposite of this attitude is that of those who, with the sole worry of not falling behind in the technological   race, unthinkingly purchase highly automated instruments (e.g. spectrophotometers or electroanalytical systems featuring built-in microprocessors) and lay aside conventional instruments which are in good working order and perfectly suited to their actual needs. It is also relatively frequent for some laboratories to purchase automatic analysers with a high throughput and capable of performing simultaneous and sequential determinations and then exploit only 10–20% of their potential capabilities.

Laboratories can be automated in one of two ways, either by purchasing new instruments or by adapting those already available with the aid of different units (modules). Which way is chosen is a matter of convenience. Thus, the determination of amino-acids in protein hydrolysates calls for a new instrument based on HPLC principles and known as a sequencer. However, in many instances, one or several of the stages of some analytical procedures can be automated by use of one of the large number of modular elements available. Thus, an FIA system fitted to a conventional photometer considerably reduces human participation in the preliminary stages (sampling, interference removal, transport to the detector, etc.). The on-line incorporation of electronic integrators or microcomputers allows the acquisition of the analytical signals provided by the detector, and also their appropriate treatment in delivering the required results with the human involvement only in the computer programming.

## 1.2 OBJECTIVES OF AUTOMATION

The objectives pursued in partly or completely automating analytical laboratories are varied. Thus, the automation of non-routine work (e.g. research) is chiefly aimed at facilitating a laboratory process that otherwise is not

feasible owing to the limitations of manual operators. Such is the case with the manipulation of radioactive materials, the need to carry out a large number of repetitive experiments in a continuous fashion or those situations where vast amounts of data are generated at a high speed or over long periods.

TABLE 1.1

Basic objectives of laboratory process automation

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. Processing of a large number of samples
. Determination of several components in the same sample
. Reduction of human participation to:
     – Avoid errors
     – Cut costs
. Increasing sample throughput
. Process (industrial or otherwise) control
. Lowering consumption of sample and/or reagent(s)
. Facilitating an anaytical technique or method

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The basic objectives of automation of the analytical laboratory, summarized in Table 1.1, tend to solve a variety of problems related to one or several of the following aspects:

(a) *Samples*, occasionally dealt with in large numbers or too scarce or valuable to be handled manually.

(b) *Analytes*, which are sometimes present in very dissimilar (macro components, traces) or low (ultra-traces) concentrations in the sample.

(c) *Reagents*, some of which are scarce or expensive (e.g. enzymes), or even unstable.

(d) *Rapidity*, frequently essential in large laboratories such as those in hospitals, urgently requiring the analytical results (e.g. clinical parameters in acute crises or shock treatments), and of industrial and other laboratories requiring constant availability of data for process control.

(e) *Economy* in personnel and material expenditure.

(f) *Precision*, closely related to the elimination of both definite and indefinite errors arising from the so-called 'human factor' (tiredness, mood, prejudice, pathological complaints and so forth).

(g) *Data generation*. Some analytical techniques are based on the acquisition of a large number of data. Even if these are generated at a rate hand-

able by a manual operator, the tedious nature of their acquisition and mani-
pulation makes it advisable to entrust them to an automated system, obviously
indispensable when data are generated at a high rate (e.g. in stopped-flow
methods).

(h) *Data processing* is better performed automatically when a large number
of data are generated by the same or many different samples, or when their
subsequent treatment is complex and liable to error if human participation is
involved in the process (e.g. in transcriptions, transfers, recordings, etc.).

(i) *Analytical technique* or *method*, occasionally unfeasible with the in-
volvement of an operator —this book abounds in illustrative examples of this
kind. Thus, electrothermal vaporization atomic absorption spectroscopy demands
the automation of the sample thermal treatment in the graphite tube via a
microprocessor programming the different heating stages involved (automation
of methodology). Likewise, the use of image detectors in spectroscopy calls
for computerized data acquisition, impossible with manual operators.


## 1.3 DEFINITIONS

The definitions given below are aimed at clarifying a series of concepts
related both to the analytical process and to its automation used throughout
this book.

The analytical literature abounds with references to different concepts,
facts or processes by the same name. It is therefore advisable to establish a
clear, hierarchical distinction of such frequently confused terms, based on
that reported by Taylor [7] and including:

(a) *Analytical process*, namely the series of analytical operations between
samples and results. It usually involves a large variety of stages which can
be summarized in three groups: preliminary operations, measurement of the ana-
lytical signal and data treatment.

(b) *Analytical technique*, *viz.* a scientific principle adapted to one or
several instruments to obtain information about diverse material and methodo-
logical aspects. Gravimetry, atomic absorption spectroscopy, coulometry, etc.
are all representatives of analytical techniques.

(c) *Analytical method.* This is the actual application of a given analyti-
cal technique in the analytical process. Thus, in gravimetric analysis, the
precipitation stage can be carried out traditionally or by precipitation in a
homogeneous solution; the atomization in atomic absorption can be effected by
aspiration of the sample solution into the flame or by electrothermal vaporiz-
ation; coulometry has two basic methodological varieties, namely constant in-
tensity and constant potential. The nature of the method also varies with the
manner in which the sample is manipulated or the data are treated. Thus, the

determination of sulphur dioxide based on the photometric monitoring of the product yielded in the reaction between the analyte, formaldehyde and p-rosaniline differs methodologically depending on whether environmental (acid rain water) or wine samples are concerned. The different ways in which the data provided by the signal-time kinetic curve can be treated give rise to as many methodological alternatives to determinations based on reaction-rate measurements.

(d) *Analytical procedure.* This term should only be used to refer to the set of precise instructions followed in implementing an analytical method and aimed at the determination of one or several particular analytes in a given type of sample. In his original hierarchical distinction, Taylor also includes the term *protocol*, subsidiary to and even more specific than procedure.
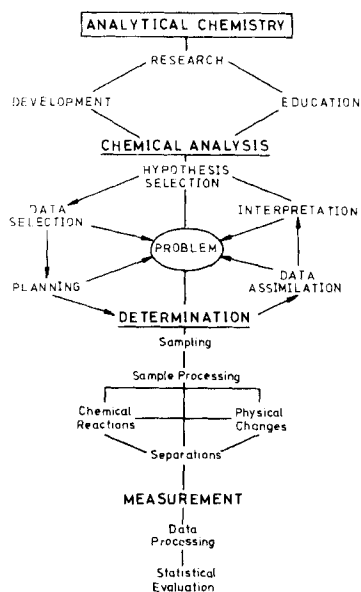


**Fig. 1.1** Major functional processes in Analytical Chemistry according to Pardue. (Reproduced from [8,9] with permission of the American Chemical Society).

'Analysis', 'determination' and 'measurement' are a triad of also readily confused terms which, according to Pardue [8,9], can also be defined in a hierarchical way. Thus, *chemical analysis* is just one of the four chief components of Analytical Chemistry —the other three are research, development and teaching. The *determination* is one of the stages of chemical analysis, which also

comprises the investigation of the unknown —one of whose components is the sample itself—, selection of the hypothesis to be applied and data to be used, interpretation of the data obtained, etc. The *measurement* is a sub-stage of the determination, which also includes sampling, separations, data acquisition and treatment, etc. Pardue's hierarchical view of Analytical Chemistry is illustrated in Fig. 1.1. According to him, samples (unknowns) are *analysed*, analytes are *determined* and parameters qualitatively or quantitatively related to these are *measured.*

The IUPAC Commission for Analytical Nomenclature laid down a series of definitions which distinguish and specify the essential features of Automatic Methods of Analysis [10]. *Not all the instruments, systems or methods designed to reduce human intervention can be regarded as automatic.* Thus, IUPAC clearly distinguishes between 'mechanization', 'instrumentation' and 'automation'. *Mechanization* is related to the production of motion and is defined as "the use of mechanical devices (machines) to replace, refine, extend or supplement human effort". A *mechanism* is "a combination of parts, of which one at least is moveable, capable of producing an effect." A *machine* or *apparatus* is a system made up of one or several mechanisms which perform one or more actions. *Instrumentation* is related to information production and transmission. An *instrument* is a device used to observe, measure or communicate a property (parameter), which replaces, refines or supplements human action. The terms 'instrument' and 'apparatus' are often used erroneously as synonyms. The essential difference between the two lies in whether or not they provide information. Thus, a centrifuge is apparatus, whereas a photometer is an instrument. While a centrifuge can indeed offer an analogue or digital read-out of its rotation speed (rpm), this information is not related to the analyte concentration. The transmittance or absorbance provided *can* be considered to be information as it is used to calculate the aforesaid concentration.

*Automation* involves the use of systems (instruments) in which an element of non-human decision has been incorporated. It is defined as "the use of combinations of mechanical and instrumental devices to replace, refine, extend or supplement human effort and faculties in the performance of a given process, in which at least one major operation is controlled, without human intervention, by a feedback system. A *feedback system* is defined as "an instrumental device combining sensing and commanding elements which can modify the performance of a given act.

According to IUPAC's recommendations, a clear distinction should be made between 'automatic devices' and 'automated devices'.

*Automatic devices* are those which "cause certain required actions to be performed at given points in an operation, without human intervention". The

system makes no decisions and the operation sequence is always the same. They possess no feedback system.

*Automated devices* are defined as those enacting automation. They are conceived to make decisions with the aid of a feedback system, without human intervention. There is a different operational sequence for each situation (sample). These systems are self-monitoring and self-adjusting, have greater independence than automatic devices and are sometimes called 'completely automatic'.

The distinctions established by IUPAC are clear-cut. Thus, the speed of titrant addition is always constant in an automatic titrator, whereas it is adjusted by a feedback system according to the nearness of the equivalence point in an automated titrator. However, some workers [11,12] acknowledge the accuracy of these definitions but consider them too stringent. Very often, the term 'automatic' is used to refer to systems with and without feedback indistinctly. In any case, whenever the concept 'automatic process' is referred to in this book, it will be meant in its widest connotation, namely that involving partial or complete elimination of human intervention not related to instrumentation.
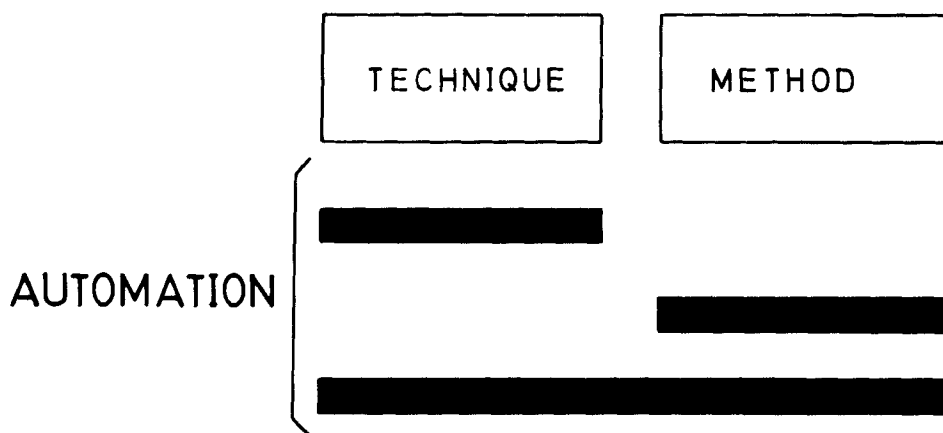


**Fig. 1.2** Ways to automate chemical analysis.

According to the definitions laid down above, both the analytical technique and the analytical method are liable to automation, either individually or jointly (see Fig. 1.2).

**1.4 AUTOMATIC ANALYSERS: CLASSIFICATION**

An *analyser* can be defined as a series of elements —modular or not—, of which at least one is an instrument, which operate with different degrees of automation and have been designed for the qualitative or quantitative determination of one or several analytes in a single or a series of samples based on changes in its physical, chemical or physico-chemical properties. It can provide results in the required form or simply offer raw data [13].

**TABLE 1.2**
Classification of analysers

| | | |
|---|---|---|
| According to the degree of automation | Automatic Semi-automatic | |
| According to the way in which samples and reagents are transported | Batch (discrete) Continuous  Robotic | Segmented Unsegmented |
| According to the number of analytes per sample | One-parameter Multi-parameter | |
| According to flexibility | Specific Flexible | |
| According to source | Commercial Home-made | |
| According to state of aggregation of sample | Gas analysers Liquid analysers Solid analysers | |
| According to foundation | Based on physical principles Based on physico-chemical principles | |
| According to sampling frequency | One-off Periodic Continuous | |

Table 1.2 shows several classifications of analysers according to different criteria. Thus, a first classification is based on whether automation is *partial* or *complete*. Because of the difficulty involved in correctly applying the terminology in this respect, establishing clear distinctions is understandably difficult on account of the variety in the degree of automation. A *(completely) automatic analyser* is defined as an analytical processor receiving one or a series of untreated, unquantized (weight, volume) samples to provide the analytical results sought in the required form without the need for the operator's intervention at any point in the intermediate stages of the process. If any of such stages is carried out manually —the commoner case—, the analyser is said to be *semi-automatic*. It should be noted that, despite its widespread use, the term 'semi-automatic' is not supported by IUPAC. This differentiation is also somewhat stringent and, in practice, the adjective 'automatic' is applied to analytical processes in which some major stage is carried out manually: such is the case with highly computerized centrifugal analysers in which the sample tray is transferred manually from the automatic dispensing unit to the reaction-measurement unit (see Chapter 8).
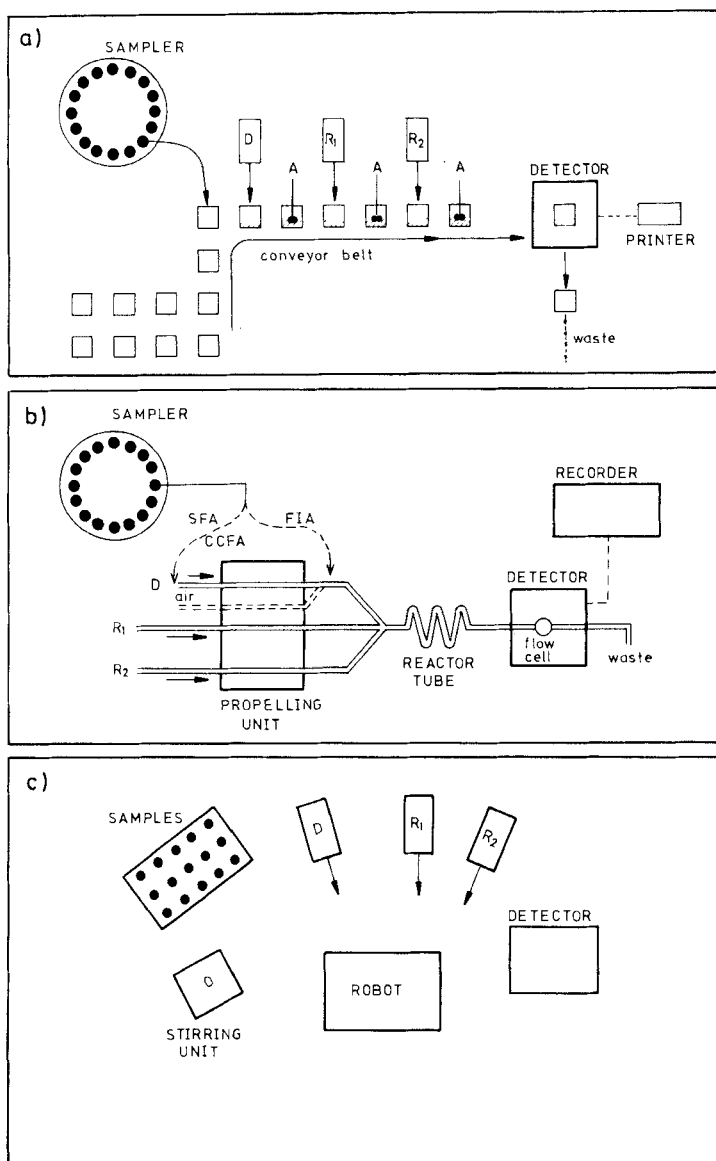
Analysers can also be classified according to the way in which samples are transported and manipulated into:

(a) *Discrete* or *batch analysers*, where each sample preserves its integrity in a vessel (cup) which is mechanically transported to various zones of the analyser where the different analytical stages (sample quantitation and reception, dilution, reagent dispensing, mixing, heating, etc.) are carried out in a sequential manner. Each sample is finally led to the detector (instrument), where signals (one per analyte) are recorded. As can be seen from Fig. 1.3a, the functioning of these analysers, described in greater detail in Chapter 8, resembles the operations carried out by a manual operator.

(b) *Continuous analysers* are characterized by the use of a continuous stream of liquid or —much less often— gas. The samples, usually liquid, are introduced sequentially at regular intervals into a channel carrying a liquid that can merge or not with other channels carrying reagents, buffers, masking agents and so on. Upon reaching the detector —generally furnished with a flow-cell—, the resultant reacting mixture yields an analytical signal which is duly recorded. This signal is transient in nature and its height or area is used to calculate the analyte concentration. The baseline between signals represents the time over which no sample zone is passing through the detector. There are two types of continuous analyser, namely:

- Segmented-flow analysers (SFA), originally developed by Skeegs [14] and first commercialized by Technicon under the name 'AutoAnalyzers', in which the flow is segmented by air bubbles aimed at preserving the integrity of samples

**Fig. 1.3** Scheme of the different types of automatic analysers, classified according to the way in which sample transport is effected. The examples illustrate the determination of a single analyte in a liquid sample requiring dilution (D) and sequential addition of two reagents ($R_1$, $R_2$) for the analytical reaction to develop. (a) Batch analyser. (b) Continuous analysers (SFA, segmented-flow; FIA, flow-injection; CCFA, completely continuous flow). (c) Robot station. Note that agitation is carried out by independent units in (a), is not required in (b) and is effected by a single unit in (c). (Adapted from [17] with permission of Ellis Horwood).

and removed prior to reaching the detector. They are discussed in Chapter 5 [15].

– Unsegmented-flow analysers (Fig. 1.3b) can be classified according to whether samples are injected or continuously inserted into the system, into 'flow-injection analysers' (FIA) [16,17] and 'completely continuous flow analysers' (CCFA) [18], respectively. Both are described in detail in Chapters 6 and 7, respectively.

(c) *Robotic analysers*, which should rather be referred to as 'robot stations', are based on the use of a high-precision minirobot whose movements mimic the actions of a human operator (Fig. 1.3c). By means of a hand (grip), the robot takes the sample and the products resulting from the different stages of its processing to a series of apparatuses (dilutor, liquid dispensing units, extractors, centrifuges, heaters) and instruments (balance, photometer, chromatograph). A single microprocessor usually controls the robot's motion and the operation of the different apparatuses and instruments, from which it receives the corresponding signals to be treated in order to obtain the final results [19]. Chapter 9 is devoted to the use of robots in the analytical process.

Depending on the number of analytes that can be assayed per sample, analysers can be classified into *one-parameter* (e.g. centrifugal and flow-injection analysers) and *multi-parameter*. The latter are of special use in clinical assays, usually requiring the determination of several parameters in blood or urine —the SMAC, an extremely powerful analyser manufactured by Technicon allows the determination of up to 20 parameters (analytes) per sample. Because of reminiscences of former times, some workers still use a parallel nomenclature (single-channel and multi-channel) to refer to these analysers. This is acceptable as the earliest commercially available continuous segmented flow analysers (Technicon AutoAnalyzers) carried out one determination per channel into which the sample was split. Hence the equivalence between 'channel' and 'parameter', exclusive to this type of analyser.

A classification of great practical interest divides analysers according to their *flexibility* for adaptation to different situations or needs (i.e. different types of sample or analyte) into 'specific designs' and 'flexible designs'.

(a) *Specific designs* are aimed at determining a single analyte or a few in the same type of sample. Their adaptation to other applications is normally unfeasible or requires major modifications. The automated assemblies for the determination of nitrogen by the Kjeldhal technique are a representative example, as are the analysers marketed by Leco for a variety of determinations: carbon and sulphur analyser (CS-244), nitrogen and oxygen determinator (TC-136) for ferrous and non-ferrous materials, etc.

(b) *Flexible designs* are characterized by their ready adaptation to different needs (types of sample or analyte) by merely changing one or several modular elements. They generally allow for changes in the reagents, configuration, detector, methodology and even the sub-stages of the process. Obviously, the flexibility of these designs will vary from one to another.

Semi-automatic analysers can also be classified according to other less relevant concepts such as the state of aggregation of the sample, the way in which the signal is measured and the sampling frequency.

(a) Depending on the state of aggregation of the sample, analysers can be classed as *gas*, *liquid* and *solid*. Obviously, the analyser design strongly depends on the type of sample to be handled. A solid analyser is usually much more complex than a liquid or gas analyser unless it is based on direct physical measurements; in fact, weighing is difficult to automate in all but robotic analysers, as are preliminary operations such as dissolution, disaggregation, extraction and so forth. The collection and treatment of liquid samples is much more affordable by most analysers. On the other hand, gas analysers are more frequently employed in industrial continuous process control and pollution monitoring.

(b) Depending on the way in which the signal is measured, one can distinguish between analysers based on *physical*, *chemical* and *physico-chemical* principles. Those based on physical properties of the sample or the analyte (e.g. density, refractive index, thermal conductivity, magnetic susceptibility) are characterized by their simplicity and by their notorious lack of selectivity; in addition, they are sensitive to pressure and temperature changes. Analysers based on chemical and physico-chemical principles are commoner and offer clear advantages over those mentioned above. The use of optical (photometric, fluorimetric) or electroanalytical (potentiometric, voltammetric) detectors among others, and also the occurrence of one or several chemical reactions, considerably increase the selectivity and sensitivity of measurements.

(c) Depending on the sampling frequency achieved, analysers can be classified as 'one-off', 'periodic' and 'continuous'.

– *One-off* analysers are conceived for sporadic determinations. They are used when the number of samples to be analysed is not too large and delivery of results is not too urgent.

– *Periodic* analysers are aimed at analysing a series of samples received at given intervals. Such is the typical case with clinical samples. They are also employed in industrial control of mass production lines involving a relatively consistent process.

– *Continuous* analysers, typically represented by 'process analysers' [20, 21] use the continuously generated results to adjust an industrial process *in*

*situ.* Environmental monitors, exposed in strategic places to send data continu-
ously to a surveillance station, are another representative example. These
analysers are described in greater detail in Chapter 16.

Finally, analysers can be classified according to their source or cons-
truction into *home-made* and *commercial.* Although there is a vast range of com-
mercial analysers available to the potential user, some workers develop their
own 'home-made' systems, which occasionally exceed the former in performance.
While FIA configurations can be readily assembled from available parts, batch
or SFA configurations are difficult to customize and are best purchased as
supplied by the manufacturers.


## 1.5 DEGREES OF AUTOMATION

As can be seen in Fig. 1.4, every analytical process consists of three es-
sential stages, namely:

(a) *Preliminary operations,* the most complex and varied of the three
stages. They include sample collection and treatment, which will vary with
the state of aggregation (dissolution or disaggregation, centrifugation, fil-
tration, gas entrapment) and the potential interference from the matrix (dif-
ferent separation techniques); the development of the analytical reaction and
the transport of the reacting mixture to the detection system.

(b) *Measurement and transduction of the analytical signal* by means of the
detector used (optical, electrochemical, thermal) and on which calculation of
the concentration of the analyte(s) is based.

(c) *Data acquisition* and *treatment.* This final stage can be implemented
with a straightforward y-t recorder or with a microcomputer which can not
only treat the acquired data and process them, but also pass them on to a cen-
tral computer governing the analytical operations of a large industrial or
hospital laboratory.

The main levels of automation in the analytical laboratory were defined re-
cently [22]. As stated above, the concept of automation is still confusedly
applied to analytical processes, techniques and methods. Therefore, against
IUPAC's recommendations, it is worth establishing different degrees of automa-
tion in order to refer more accurately to the extent of replacement of human
intervention in the laboratory. According to this criterion, analytical pro-
cesses can be classified into (Fig. 1.4):

(a) *Semi-automatic* (I), namely those with *one* automated stage. There are
five types of analyser used to implement this type of process.

(b) *Semi-automatic* (II). Processes with *two* stages requiring no human in-
tervention. They are carried out by three types of analyser.

(c) *Completely automatic*, where the analyser —commonly given the name 'analytical black box' [23]— totally replaces the human operator.

The differences between the nine types of analyser referred to above are not clear-cut; in fact, the scheme in Fig. 1.4 is not exhaustive and could be expanded —yet, it is representative of automation in Analytical Chemistry. Below are described the nine alternatives to automation following the scheme in Fig. 1.4.

ANALYTICAL  PROCESS
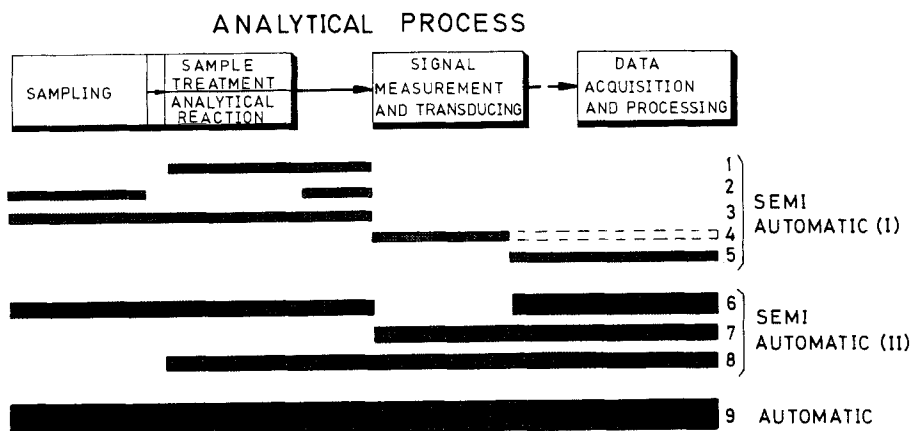


**Fig. 1.4** Degrees of automation of the analytical process, assumed to consist of three analytical stages. Numbers 1 to 9 denote the different levels at which human intervention is replaced.

*Type 1.* This involves automation of the developent of the analytical reaction and the transport of the reacting plug to the detector (generally continuous in nature and furnished with an optical or electroanalytical flow-cell. A representative example of this alternative is presented in Fig. 1.5, namely the flow-injection determination of aluminium in silicate rocks based on the formation of a coloured chelate between the metal and Xylenol Orange ($\lambda_{max}$ = 560 nm) [24]. The sample collection and dissolution (disaggregation) stages, which are tedious, are done manually, as is the injection of an accurately measured volume of treated sample. However, the main analytical reaction and removal of interferences (addition of ascorbic acid to reduce $Fe^{3+}$ to $Fe^{2+}$ and EDTA to form soluble chelates with a large number of potentially interfering metal ions) are carried out in a continuous fashion, as is the transport to the photometric detector used. An ordinary recorder acquires the transient signals yielded upon passage of the reacting plug through the flow-cell. Data treatment is also performed manually.
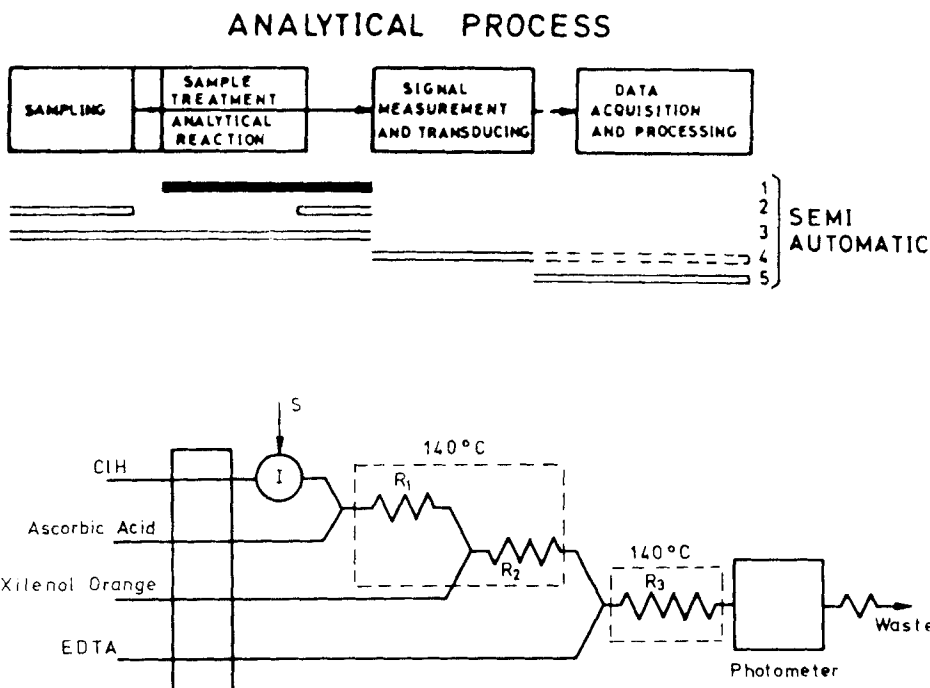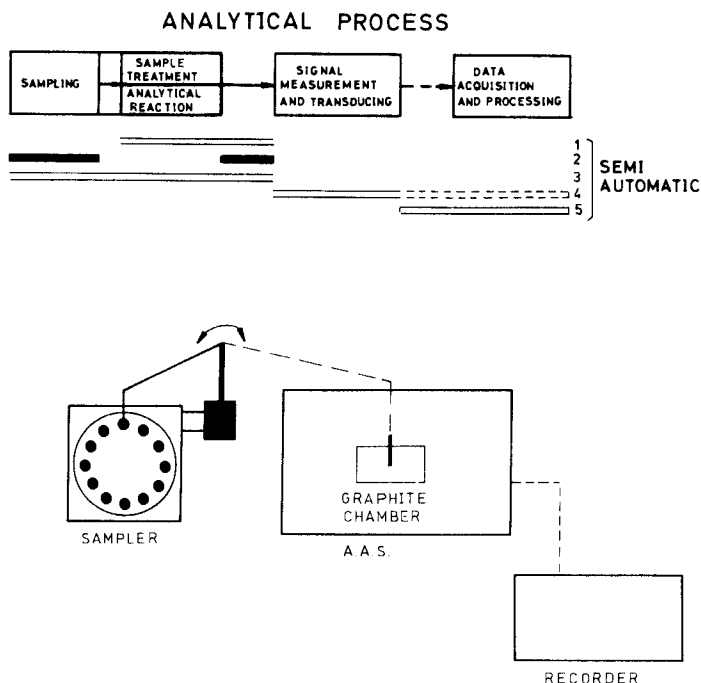
# ANALYTICAL PROCESS



**Fig. 1.5** Automation of the first few stages (preliminary operations) of the anlytical process in a Type 1 analyser, an FIA assembly for the determination of aluminium in rocks. (Reproduced from [24] with permission of Pergamon Press).

*Type 2.* This involves partial automation of the first stage of the analytical process: the accurate measurement of a sample volume (sampling) and its transport to the detector without human intervention. However, sample treatment (e.g. dissolution) and the analytical reaction development —if required— are carried out manually. Figure 1.6 shows a representative example: the incorporation of an automatic sampler in a thermal-vaporization atomic absorption spectrometer. This instrumental configuration is representative of those where the automation of one stage is highly recommendable —in this instance to ensure reproducible results.

*Type 3.* The implementation of all the preliminary operations in the analytical process without human intervention represents a remarkable degree of automation. By incorporating a sampler in ordinary FIA assemblies or the classical AutoAnalyzers, the first stage of the analytical process could be regarded as automated. However, it should be noted that the sampler holds pre-treated samples, so that the automation of the first stage is only apparently complete.
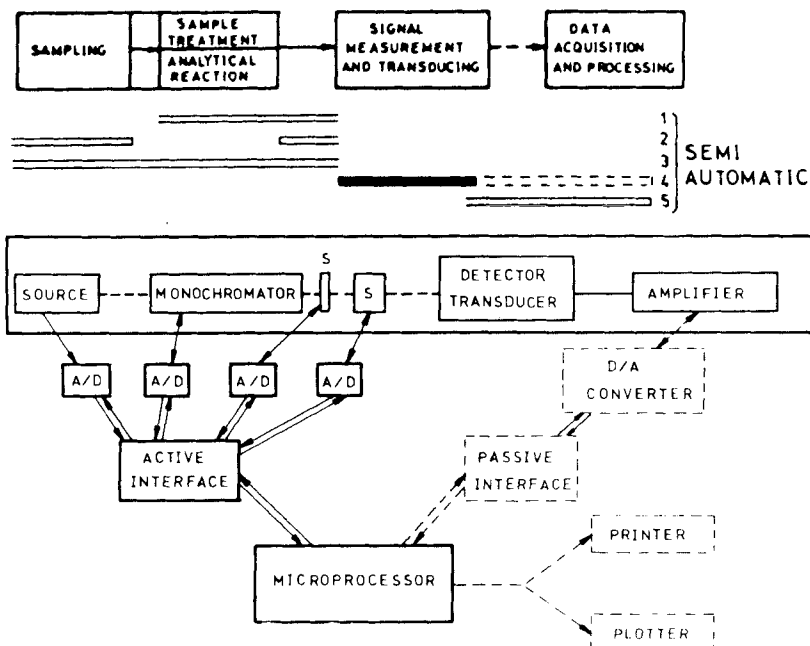
## ANALYTICAL PROCESS





**Fig. 1.6** Automation of the first few stages (preliminary operations) of the analytical process (Type 2 analyser). Automatic system for introduction of samples in electrothermal-vaporization atomic absorption spectroscopy.

*Type 4.* The automatic control of an instrumental analytical technique — whether optical, electroanalytical, magnetic or thermal— by means of a microprocessor has become commonplace in commercial instruments in the last few years. Thus, the control of the parameters governing the functioning of a conventional molecular absorption spectrometer (lamp selection, monochromator movement, change of slit width, movement of the cells in the measuring compartment, etc.) is carried out via the keyboard of a microcomputer linked to the system through an active interface. Figure 1.7 depicts an example of automation of the second stage of the analytical process. However, this situation is currently uncommon as the microcomputer used can also deal automatically with data acquisition and treatment as in Type 7 analysers.

*Type 5.* This involves the automation of data acquisition and treatment in a traditional analytical instrument —even a balance can be the subject of automation. As shown in Fig. 1.8, a microcomputer connected on-line with the analogue output of the instrument ensures the automation of this stage of the
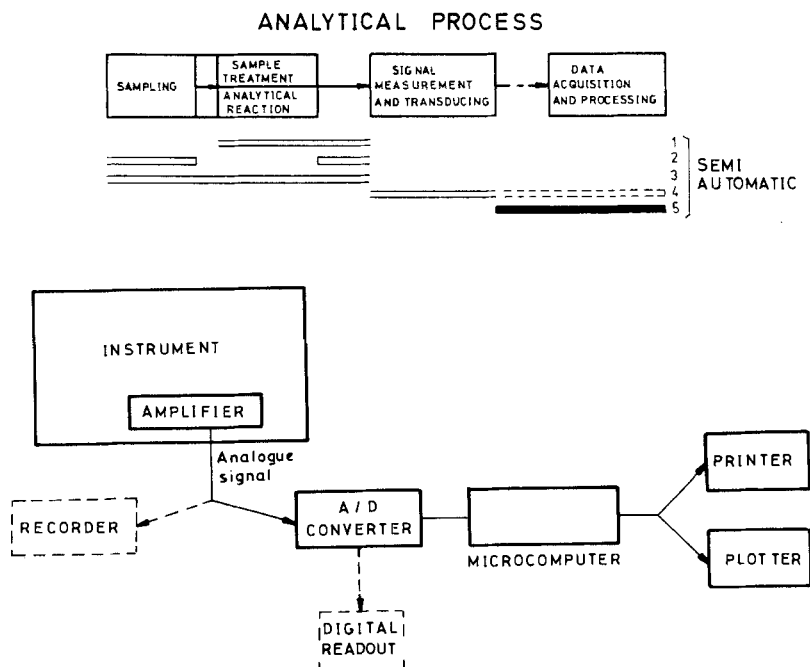
# ANALYTICAL PROCESS



**Fig. 1.7** Automation of the second stage of the analytical process (Type 4 analyser). Use of a microprocessor incorporated in a molecular absorption spectrometer to control its functioning through an active interface and an analogue-to-digital converter.

analytical process. An A/D converter allows data to be entered into the computer and read on a digital display. The computer can be programmed to deliver the results via a printer or plotter, as required. Note the difference between the data provided by a y-t recorder (non-programmable) and those offered by a computer-programmed plotter. This alternative to automation is essential when data are generated at a high rate (e.g. by spectrophotometers with image detectors or in stopped-flow methods applied to ultra-fast kinetics) or to avoid sample-result mismatching when dealing with a very large number of samples. It is also recommendable when the data are to be subjected to a complex mathematical treatment (measurement of areas and resolution of overlapping peaks in chromatography, differential kinetic methods, etc.). In short, the aim is to relate the acquired data to the analyte concentration by means of a previously run calibration graph or straightforward statistical computations.

*Type 6.* The previous alternatives involved the automation of a single stage of the analytical process. Type 6 analysers eliminate human intervention

ANALYTICAL  PROCESS



**Fig. 1.8** Automation of the third stage of the analytical process (Type 5 analyser). On-line incorporation of a microcomputer for data acquisition and treatment.

in two stages: preliminary operations and data acquisition and treatment. This type of analyser uses a conventional sensing system whose functioning is governed manually. However, this stage is also —apparently— automated as, once the working parameters have been selected, the samples are introduced into the analyser and the results are delivered without the operator's intervention, which is required only whenever the sample or analyte to be determined or the detector settings are to be changed. A typical example of this level of automation is an automatic analyser for the determination of pollution levels in waste water, based on reversed FIA principles [25], the scheme of which is shown in Fig 1.9. The waste water is continuously introduced via a peristaltic pump which also fills the loops of the injection valves with the reagents. A microprocessor controls the functioning of the pump and the injection-reaction unit via an active interface, and acquires data from the photometric detector, which are processed and delivered through a printer.

*Type 7.* This type of analyser is employed in the automation of the second (functioning of the detection system) and third (data acquisition and treat-

ment) stages of the analytical process. As stated above, this alternative is currently available in most of the recently introduced commercial analytical instruments. Figure 1.10 shows the essential elements of an analyser of this type, namely a microprocessor furnished with active and passive interfaces via which the aforesaid stages are controlled and a printer or plotter whereby the results are delivered. Samples are introduced manually into the analyser.
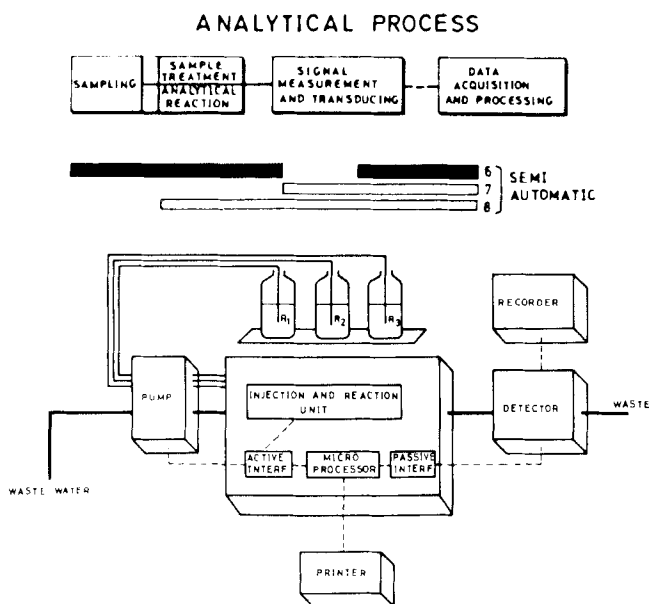
**Fig. 1.9** Automation of the first and third stages of the analytical process (Type 5 analyser). Scheme of automatic continuous analyser for determination of pollutants in waste water, based on a reversed FIA configuration. (Reproduced with permission of the copyright holders).

*Type 8.* Gas and liquid chromatographs, the most representative examples of this type of analyser, occupy a prominent place in current analytical instrumentation. Their sampling operation (injection) is generally manual and column separation can be considered to be the only sample treatment. They carry out detection in a continuous fashion and usually employ an electronic integrator or a microprocessor to acquire and process data. Their automation, therefore, involves the complete second and third stages, in addition to part of the first. The instrument's microprocessor can control: (a) the chromatographic furnace, which works at a fixed temperature in liquid chromatography and over

a given temperature gradient in gas chromatography; (b) the mobile phase gradient system in liquid chromatography; and (c) the detection system —less often. The incorporation of a sampler synchronized with an automatic injection system results in a system close to Type 9. Chapter 11 is largely devoted to the automation of chromatographic processes.

# ANALYTICAL PROCESS
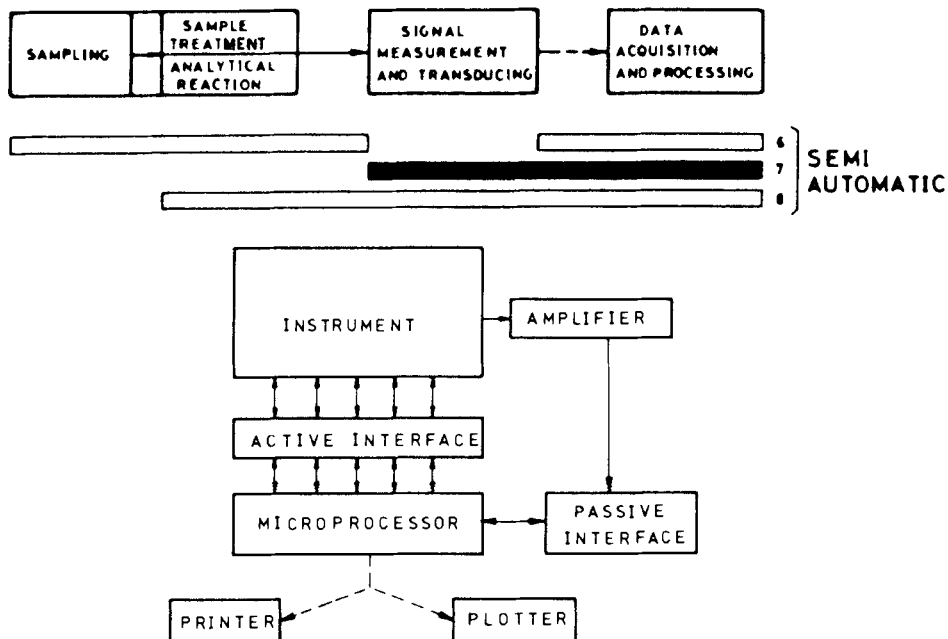


**Fig. 1.10** Automation of the second and third stages of the analytical process (Type 7 analyser). Scheme of instrument with built-in microprocessor.

Microprocessor-controlled third-generation automatic titrators are also a representative example of Type 8 analysers (Fig. 1.11). Their sensing system (a potentiometric or photometric probe) is manually controlled, as is sample changeover in the titration zone. Titrant addition (analytical reaction) is governed by the microprocessor: the flow-rate changes with time as a function of the position on the titration curve (it is lower in the vicinity of the equivalence point). The microprocessor collects data from the burette and the measuring system and delivers results in the required form (a titration curve, the analyte concentration). Systems using a y-t recorder instead are representative of a lower degree of automation and fall outside this general scheme. Conversely, the incorporation of an automatic sampling system (e.g. a

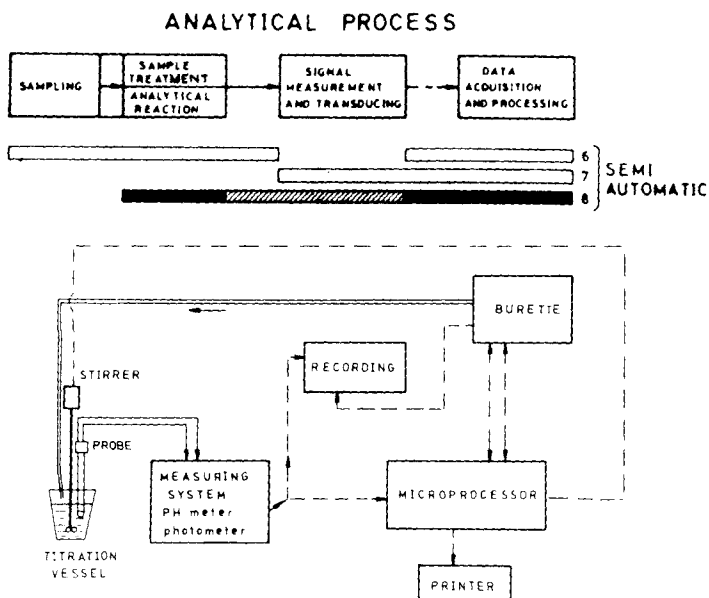Mettler or Metrohm sampler) results in a Type 9 configuration with some limitations (see Chapter 13).

## ANALYTICAL PROCESS



**Fig. 1.11** Automation of the first and third stages of the analytical process (Type 8 analyser). Scheme of microprocessor-controlled automatic potentiometric or photometric titrator.

*Type 9.* This is represented by the above-mentioned completely automatic analyser, where untreated samples are introduced without quantitation into the instrument, which provides results in the requested form with no human intervention other than programming the analyser (Fig. 1.12). The few commercial and custom-made analysers that fall into this category are used to meet very specific needs.

The concept 'completely automatic' is not strictly accurate. In fact, some of the analysers labelled as such require human intervention in or control of one sub-stage.

Undoubtedly, preliminary operations make up the stage that is most difficult to automate on account of the variety and complexity of the different sub-stages involved. In addition, the concept of 'sample' is applied to different situations. Strictly, it should be used to refer to the raw material with which the operator or analyser is initially confronted, namely urine or whole

blood from a patient, water collected at a given depth in a lake, coal in
lumps of different sizes and so forth. Sample collection and treatment (e.g.
transfer of urine to a sampler vial, blood deproteination, collection and stor-
age of a water sample, coal homogenization, etc.) are difficult to automate
with the exception of a few cases such as *in vivo* clinical controls or the
continuous collection of water samples introduced directly into the analyser.
From all this it follows that complete automation, as presented in Fig. 1.12,
is an elusive goal. Robot stations, described in Chapter 9, probably represent
the closest situation in this respect.

## ANALYTICAL PROCFSS
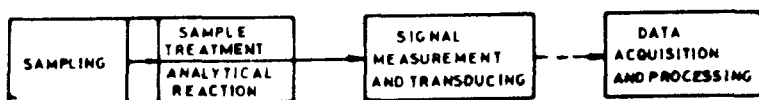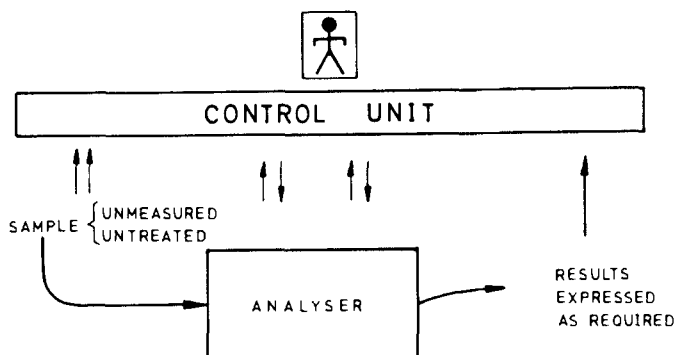


**Fig. 1.12**  Complete automation of the analytical  process (Type 9 ana-
lyser).

The automated FIA system classed as a Type 6 analyser can be regarded as
completely automatic in spite of the fact that the detector parameters are man-
ually controlled, which does not really involve human intervention in the ana-
lytical process provided that the same type of sample and analyte(s) is dealt
with throughout.

Centrifugal analysers, discussed in Chapter 4, are discrete in nature. Sam-
ple collection and reagent dispensation take place in an automated dosing mod-
ule. However, the transfer disc containing the radially arranged samples and
reagents is transferred manually to the analyser module, where reaction, sig-
nal measurement and data acquisition and treatment, all completely automated,

take place. As can be seen from Fig. 1.13, this type of analyser is not com-
pletely automatic as claimed by manufacturers, insofar as one of the intermedi-
ate stages has to be performed manually. This situation is different from that
described above as the analytical process would be unfeasible without the
operator's continuous intervention.

## ANALYTICAL PROCESS
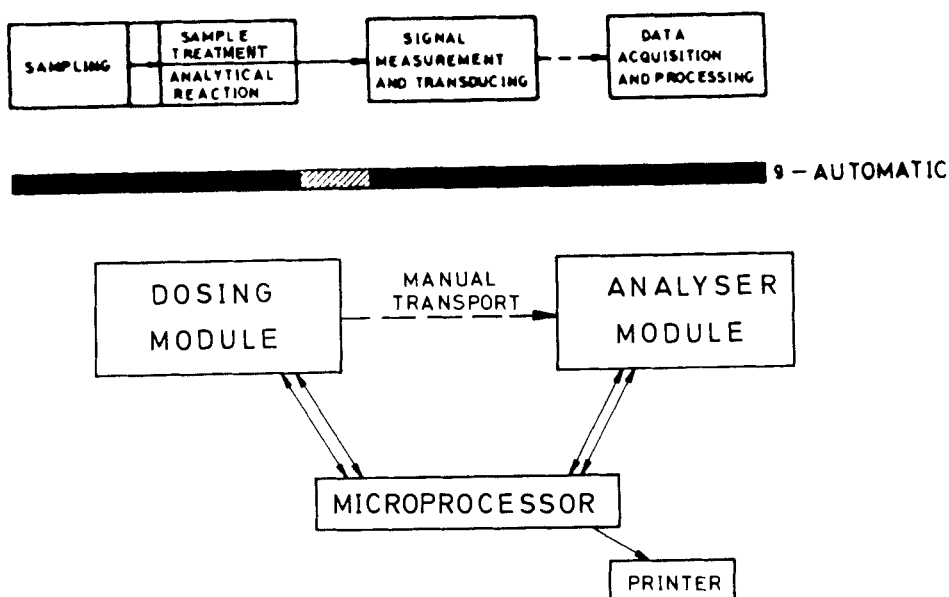


**Fig. 1.13** Seemingly complete automation of the analytical process
(Type 9 analyser). Centrifugal analysers feature an intermediate stage
involving the manual transport of the transfer disc.

## 1.6 ANALYTICAL TECHNIQUES AND AUTOMATION

From the point of view with which the automation of analytical instrumenta-
tion has been approached above and taking into account the distinction between
analytical technique and method established in this chapter, analytical deter-
minative techniques and automation can be related in two ways.

(a) A given analytical instrumental technique can be inherent in an auto-
matic analyser. Such is the case with optical (photometry, fluorimetry) or
electroanalytical (potentiometry, amperometry) techniques, which can be imple-
mented by means of self-contained modules on different analysers which are
thus endowed with different degrees of automation —in the second stage of the
analytical process in this instance. On the other hand, an analytical tech-
nique can be use to automate an analyser functioning in addition to sample

acquisition and tretament, as with instruments featuring built-in micropro-
cessors (Type 3 analysers, Fig. 1.7).

In Table 1.3 are listed the instrumental techniques most frequently used
for detection with automatic analysers of any kind. Molecular absorption spec-
trometry (colorimetry) is by far the commonest of such techniques, followed by
potentiometry (pH measurements and use of selective electrodes).

**TABLE 1.3**

Analytical determinative techniques most frequently used in automatic  methods
of analysis

| Spectroscopic | Molecular | Absorption | UV-visible | Colorimetry |
|---|---|---|---|---|
| | | | | Photometry |
| | | | IR | |
| | | Emission | Fluorimetry | |
| | | | Chemiluminescence | |
| | | | | |
| | Atomic | Refractometry | | |
| | | Absorption | AAS | |
| | | Emission | Normal | |
| | | | ICP | |
| | | | | |
| Electrochemical | Potentiometry | | | |
| | Conductimetry | | | |
| | Voltammetry | | | |
| | Amperometry | | | |
| | Coulometry | | | |
| | Dielectric constant measurement | | | |

A given instrumental technique can be considered to bear different rela-
tionships to different laboratory processes. Thus, spectrofluorimetry can be
automated by use of a microprocomputer: either by setting the desired instru-
mental parameters (excitation and emission wavelength, slit width, instru-
mental sensitivity, etc.) or by choosing the methodology (conventional, syn-
chronous, synchronous-derivative, time-resolved, etc.) to be used. Automated
data acquisition allows the implementation of these alternatives (see Chapter
9). On the other hand, a fluorimeter equipped with a flow-cell can be used as

a modular add-on to a continuous analyser. Thermal techniques, in contrast, are seldom used with automatic analysers; yet, there is a variety of commercially available instruments such as thermobalances, differential calorimeters and differential thermal analysers affording thermal analysis with a greater or lesser degree of automation.

As far as separation techniques are concerned, they can be implemented on automatic continuous analysers or robot stations as ancillary modules (dialysers, ion exchangers, liquid-liquid extractors). As stated in Chapter 12, chromatographic processes —particularly column (HPLC and GC), but occasionally also planar chromatographic purposes— are commonly the subject of automation. A conventional chromatograph furnished with a system for sequential introduction of samples —which can even be partially treated in a continuous fashion before of after column separation (derivatization and post-column techniques)— markedly resembles continuous flow analysers. Gas and liquid chromatographs are often used as separative-determinative modules in robot-staions.

## 1.7 DISADVANTAGES OF AUTOMATION

The outstanding advantages offered by the automation of laboratory processes were clearly illustrated in Section 1.2 in dealing with its objectives. Despite its advantages, the partial or complete elimination of human intervention in such processes may result in a series of major and minor disadvantages which should be taken into account in deciding whether a give process is to be automated or not.

The first shortcoming of automation is that the more automated the process is, the less is the contact of the chemist or worker with it. This, in turn, results in a lack of continuous control over each situation (sample) and of discrete observations increasing the knowledge of the experimental events —this prevents the operator from obtaining a certain type of information which in some cases might be even more interesting than that arranged to be obtained. In addition to this separation, the analyser requires more frequent check-ups and there is a greater risk of sample-result mismatching.

The popularity —or even magic— of replacing human effort and faculties in the realization of any task negatively affects the attitude of laboratory principals, who can overestimate the real potential of automation, falling in overconfidence that may adversely influence the planning of some analytical operations carried out in the laboratory. As a rule, potential purchasers do not have a good knowledge of the characteristics and possibilities of the vast range of commercially available analysers and instruments. Nor do they seem to

take the trouble to study either of these aspects with the required calm and in the expected depth. To make matters worse, manufacturers tend to overpraise their products and usually make no mention of their limitations, the knowledge of which is as important as that of their potential.

The ease with which results can be generated and the above-mentioned over-confidence can also result in non-critical evaluation of the results, the sole responsibility of the chemist according to Pardue (see Fig. 1.1). The chemical 'sense' should prevail over the data delivered by the computer. If this gives a pH of 22.3 after the pertinent measurements and data treatment, one can readily spot the deviation from fact. However, if the result falls within the accepted sensible range, it is the human who must check whether such a result is consistent with predictions or further experiments. Analytical Chemistry does not end in the printer or plotter.

The automation of an analytical techniques occasionally results in  a narrowing of its scope, particularly in relation to research work, about which manufacturers do seem to worry too much. Later automated instruments, despite their changed face —a keyboard and screen have replaced a series of buttons and dials— are almost always exclusively designed for routine determinations, so some of the usual performance of earlier models has been lost in the take-over. In addition, the quality of some of the elements of the instruments is sometimes sacrificed in order not to raise costs too much by the incorporation of microprocessors. This, in turn, may result in decreased lifetimes and performance (e.g. poorer spectral resolution), and in increased maintenance.

## 1.8 SIGNIFICANCE AND SCOPE OF APPLICATION OF AUTOMATION

The growing trend towards automation has brought about a substantial change in laboratory work, not only because of the purchase of new analysers, instruments or modules, but also because of the profound alterations undergone by the operator's mission. Thus, the computer is now an indispensable tool —it can be said to have become the main link between the analyst and his environment. Laboratory staff have had to be retrained to face the new situation. This transformation results in doubtless advantages (economy, rapidity, reliability, increased potential), commented on above. Automation is of particular significance with respect to three aspects, namely:

(a) *Analytical instrumentation.* The  automated control of various instrumental parameters and of data acquisition and treatment considerably improve the performance of non-automated instruments. Such is the case with the automatic sampler used in electrothermal-vaporization atomic absorption spectroscopy (Fig. 1.6), which results in substantially improved reproducibility, or

computerized data acquisition and processing systems, which endow some methodologies and techniques with outstanding reliability and rapidity. Moreover, automation has facilitated the development of new instrumental modes (e.g. spectroscopic techniques using image detectors, Fourier transform spectroscopy, timed-resolved fluorimetry, etc.).

(b) *Non-routine work* in research and development laboratories, to which automation is extremely useful. On the one hand, it increases the analytical potential in allowing the development of new, more powerful instrumental alternatives (e.g. ultra-fast kinetics), as stated above. Spectral pattern recognition systems based on comprehensive programs running on large computers have remarkably improved the reliability of the structural elucidation of both organic (IR, mass spectrometry, NMR) and inorganic (X-ray techniques) species. It is also worth emphasizing the great aid of computers in dealing with large sets of data requiring complex mathematical calculations. The computerized optimization of laboratory processes is an invaluable tool in developing methodologies requiring fewer experiments, of great interest when these are slow or involve valuable samples.

(c) *Control laboratories.* Automation is of relevance to control laboratories aimed at ensuring the quality demanded by areas of great social interest. One such area is Clinical Chemistry. Present-day medicine bases its diagnoses on the results of a battery of tests on biological samples (urine, blood, tissues) and places less reliance on clinical experience and judgement. Since health is one of the major concerns of our society —as reflected in the social security budget of any developed country— it is easy to understand the imperative need for hospitals to obtain a large number of analytical data as quickly and cheaply as possible. Automation is an essential contribution in this context.

The situation is much the same in most of industry, where quality control laboratories have become much more important than they used to be a few years ago. The demands in this area are dictated by the large number of samples to be analysed, the need to automate manufacturing processes requiring one-off determinations at some point along the production line and the quality now required of manufactured goods. It is therefore necessary to control not only the raw materials, but also the intermediate and end products.

Biotechnology is one of the most influential sciences at the end of this century and the analytical control of biotechnological processes in laboratories and pilot and industrial plants is therefore essential. Automation is a boon to the five analytical techniques most frequently employed in this field, namely electrophoresis, immunoassay, chromatography, protein and DNA sequencing, and molecular structure elucidation.

Society's increasing awareness of ecological problems has spurred the development of automated analytical methods for monitoring pollution levels in air and in all types of water, in urban, industrial and natural environments. A consciencious study of alterations to the ecosystem by man demands the strict control of a large variety of parameters at a high rate, which is completely impossible without a high degree of automation.

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# 2
## Computers in the laboratory

## 2.1 INTRODUCTION

The already massive use of computers in household and corporate environments is still and will predictably be on the increase in years to come as an obvious result of their progressively falling cost. Laboratories have not failed to recognize their vast potential and have exploited them in a number of fields, particularly in the automation of a variety of processes. Strictly, the role of (micro)computers in process automation involves the action of electronics or hardware on the mechanical parts of an instrument with the aim of fully or partly replacing human intervention.

Despite the possibility of controlling some instruments or processes without the aid of computers, these usually result in a higher degree of automation and become a sort of 'brain' governing the system concerned. Put simply, a computer is a system capable of capturing, acquiring or accepting information from the outside world, converting it into intelligible information to be handled on its inside, making decisions on the basis of the processed input data, storing the acquired information or processing it on its own or alongside other stored information, and actuating the instruments or machines involved in a given process. Each of these stages is carried out by a different part of the computer (Fig. 2.1). The *central processing unit* (CPU) or microprocessor is the key part of the computer as it functions to make the required decisions and excute them —information processing. Every computer features two other essential elements, namely the memory and the input/output (I/O) unit. The *memory*, expandable by means of additional modules, is in charge of the storage and preservation of the acquired information (commands, addresses or data) and is characterized by its capacity, measured in kilobytes (1 kb = 1024 bytes), and according to its nature, ROM (*read only memory*) or RAM (*random access memory*). The I/O unit(s) link(s) the computer with the outside world and allow data acquisition and process or instrument control —key aspects to laboratory automation— through appropriate interfaces.

Computers can be incorporated into the laboratory in either of two chief ways (Fig. 2.2):
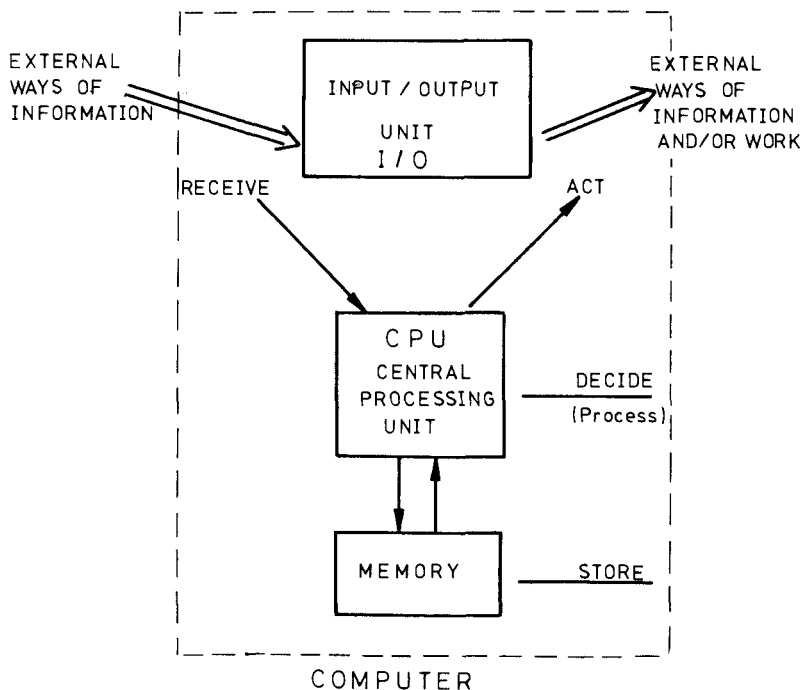
**Fig. 2.1** Scheme of a computer showing the essential function per-
formed by each of its parts.

(a) *Off-line.* The operator writes the software, carries out the experi-
ment, tabulates the results obtained and inputs them into the computer's mem-
ory as the program requests them. The computer thus only executes the software
and delivers the processed results to a display or printer. The operator is
the active part and centre of the process, and no direct link is established
between the experiment and the computer.

(b) *On-line.* The experiment and the computer are closely linked to each
other through an electronic interface. In this case, the computer not only pro-
cesses and displays data, but also acquires it from the experiment or measur-
ing instrument and controls it via interfaced electronic or electromechanical
elements actuated by voltage or current changes. On-line configurations are
the more interesting from the point of view of laboratory automation, to the
point of becoming the only viable alternative in some cases (e.g. when data
must be acquired very rapidly or the experiment generates a vast amount of
information). In addition, they feature a number of advantages over their
off-line counterparts, namely: the operator is relieved from the key role in
the process and its function is undertaken by an electronic interface, thereby

avoiding human errors; the computer acquires information directly from the experiment and hence at a much higher frequency; the computer controls the experiment in a faster and safer way than the operator does; the overall process is therefore faster and more precise. On the other hand, on-line configurations require checking of the functioning of the interface, which should be suitable for the purpose and as regards serial or parallel data transfer, conversion frequency and so forth.

a). Off-line



b). On-line
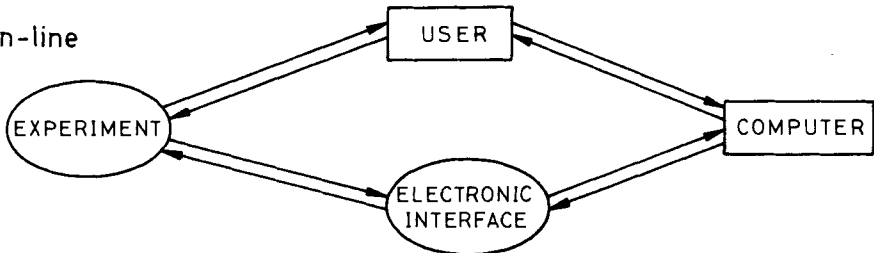


**Fig. 2.2** Ways of incorporating computers into the laboratory.

Hence, computers allow the ready automation of laboratory processes such as data acquisition and treatment, result delivery and process control. This great potential is further increased by the possibility of linking computers to one another (intelligent instruments) and by the use of workstations, expert systems and data banks, all of which are commented on in some detail below.

## 2.2 DATA ACQUISITION AND PROCESSING. RESULT DELIVERY

These three operations can only be automated with the aid of a (micro)computer. Figure 2.3 illustrates schematically the participation of the chief elements of the computer in the automation of these operations. The measuring instrument or device obtains analogue information from the experiment and the measured parameters are acquired by the computer via a suitable interface consisting of an analogue-to-digital converter (ADC) which transforms the analogue experimental signal into digital information —the only type the compu-

ter can store and handle. Interfaces used for data acquisition only are known
as *passive interfaces* insofar as they merely transduce and transfer informa-
tion from the measuring instrument to the computer. The digitized information
generated can be processed or stored in memory —usually expanded memory— for
future use. Results can be displayed in a variety of ways by fitting different
peripherals (CRTs, printers, plotters) to the computer output.
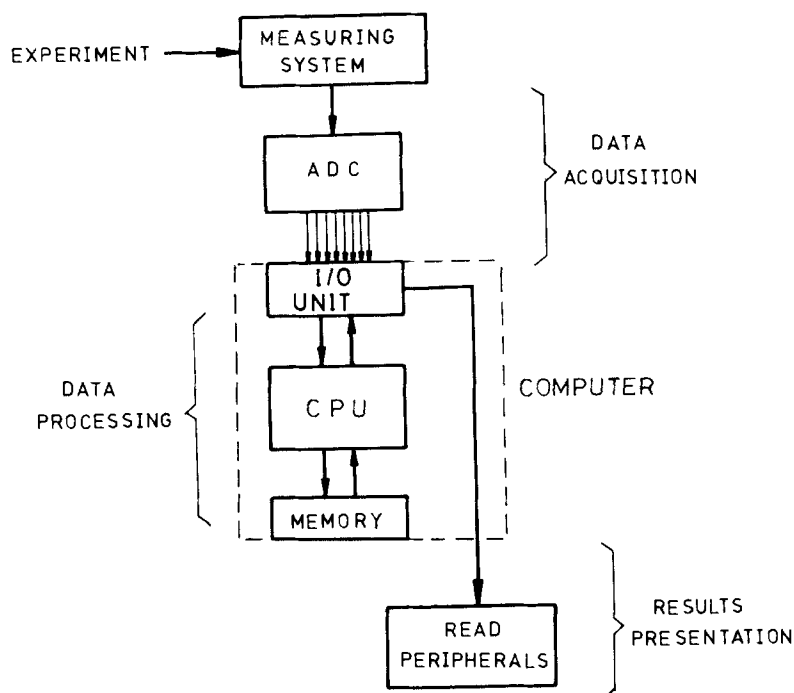


**Fig. 2.3** Scheme of computerized data acquisition and processing and
result delivery.

From the elementary scheme in Fig. 2.3 it is apparent that data acquisi-
tion is the stage lending itself most readily to automation as it establishes
an on-line link between the instrument and the computer. It is indeed an im-
portant, delicate operation. The automation of data processing and result de-
livery is ensured by the sole presence of the computer and the use of appro-
priate software.

**2.2.1 Data acquisition**

According to Barker [1], every measuring instrument liable to automation
features three essential components, namely the detector proper, the detector
electronics and the display system. The signals generated by the measuring

instrument can be sampled on output at any of these three elements, although those emerging from the detector electronics are the easiest to handle.

Many laboratory instruments currently available are supplied ready for interfacing at all three levels. It is therefore the user who decides which of the meter outputs is to be used. Although interfacing is normally effected at the second level, signals are occasionally read from the display system and stored for future use. Data acquisition at this level is of great importance when the measuring instrument generates data at a high speed (e.g. study of transient phenomena or reactions with fast kinetics) or the variation of the signal over time rather slow. Data storage is not an end in itself, but rather an intermediate step in the process of data processing.

Once the level at which signals are to be sampled is reached, they are digitized and transferred to the computer. The passive interfaces performing such a task consist of an analogue-to-digital converter (ADC) which transduces the analogue signal —usually a voltage— to the digital information acquired, processed and/or stored by the computer. There is a large variety of ADCs available [2,3]; yet, successive-approximation converters [4], with conversion times of the order of 10 μs and —unlike in counter converters— independent of the magnitude of the analogue input voltage, possess the most desirable features. As a rule, the digital ouput of these converters is 8 or 12 bits (256 or 4096 possible binary states overall) and yield good resolution, provided that a suitable sampling frequency is chosen. It should be noted, however, that analogue to digital conversion always results in the loss of some information which is only outweighed by the advantages offered by the computerized treatment of such signals.

As the computer receives digital data corresponding exactly or approximately to an analogue (experimental) signal at given —discrete— times, a *sampling frequency* consistent with that of the experimental signal must be established in order to avoid errors arising from a lack of information. Too high a frequency should also be avoided, however, as it would result in a data acquisition speed beyond the converter's capacity and hence require the incorporation of analogue multiplexers into the interface. There is the added risk of rapidly saturating all the available memory —particularly with software-controlled data acquisition— even before the experiment or its chief phase is concluded. A rough knowledge of the magnitude of the frequency with which the experimental signal will be generated is therefore mandatory in order that the sampling frequency can be matched to it.

Accurate signal sampling reportedly requires using a data acquisition speed at least double —although, preferably ten times— Nyquist's frequency (*viz.* the minimum sampling frequency). Figure 2.4 illustrates how a given sam-
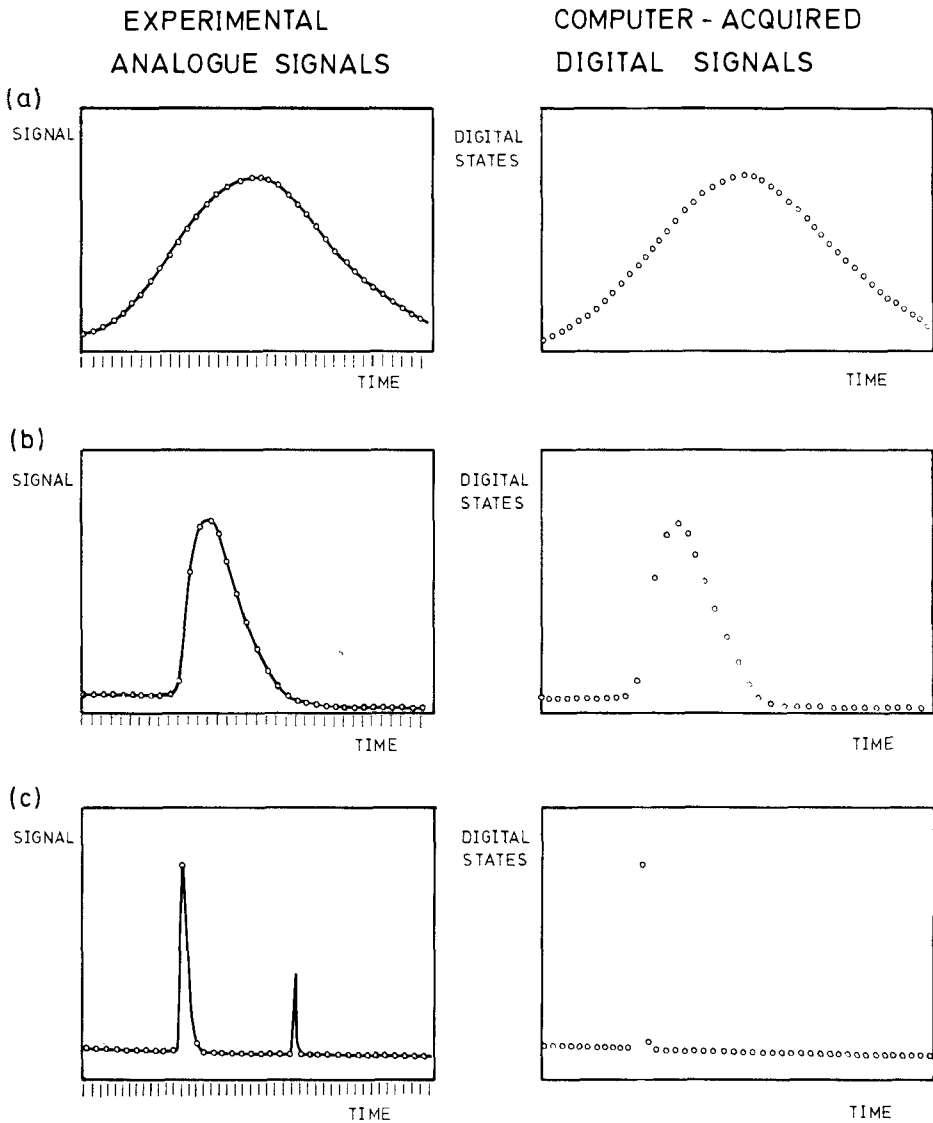
**Fig. 2.4** Result of using a too high (a), adequate (b) or too low (c) sampling frequency in the computerized monitoring of three transient signals. (The dashes below the x-axis indicate the sampling points).

pling frequency can be adequate or low or high depending on the frequency at which the experimental signal is generated. Occasionally a given experiment provides signals of very different frequencies [1]. Such a process should be sampled by using two memory buffers and auxliary memory to store the whole data set. The digitized data from the first signal are temporarily stored in the first buffer and transferred to the auxiliary memory once converted; meanwhile, the digitized data from a second signal are stored —also temporarily— in the second buffer as the first is cleared and made ready for receiving new data.

The use of adequate sampling frequencies suited to the nature of the signal concerned has been studied by a number of workers [3,5,6]. Some processes such as the control of chromatographic peaks or transient signals with baselines stable over long time intervals do not strictly call for the storage of the sampled data, but simply for a computation program ensuring that only those signal values exceeding a given magnitude representative of the baseline are stored in memory. On the other hand, Leyden *et al.* [7] used a controlled-voltage oscillator determining the appropriate sampling frequency to be used. Unlike in the previous case, it is the computer's hardware rather than the software which controls data acquisition here.

So far only sequential data acquisition has been dealt with. However, it is deed possible to receive two or more experimental data at the same time and thus perform simultaneous data acquisition. The signals to be simultaneously controlled can be obtained from various instruments of the same (e.g. gas chromatographs) or different nature (e.g. spectroelectrochemistry), or from a single instrument such as an image detector, consisting of a large number of sensing elements (e.g. diode arrays) supplying information simultaneously. Simultaneous data acquisition, of increasing importance to laboratories, requires the use of various interfacial elements (Fig. 5). The analogue signals from the sensing instruments or their elements are transferred to *sample-and-hold* (S/H) amplifiers which retain the signal (voltage) from each instrument at time t. An analogue *multiplexer* (MPX) sequentially transfers the amplified signal (AMP) from each S/H amplifier to the ADC where it is converted to digital form and read in parallel by the computer. Note that, although the signals held in the S/H amplifiers are read sequentially, the information they provide corresponds to the same instant (i.e. it is generated simultaneously).

It is therefore obvious that computerized data acquisition involves a number of essential factors such as the length of the experiment, the magnitude and frequency of the generated signals, the speed of data acquisition and the nature of the measuring instrument, all of which have been given due consideration in the literature [3,8–11] and in reports on the use of digital

multimeters [12] and voltmeters [13], oscilloscopes [14], various logic ele-
ments [11], I/O boards [15] and different commercial and home-made modules.
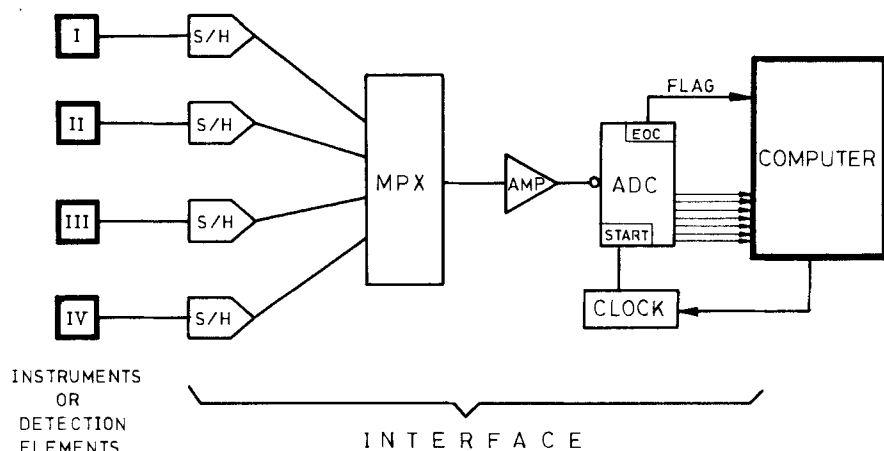


**Fig. 2.5** Simultaneous computerized data acquisition. The S/H ampli-
fiers in the interface allow the simultaneous acquisition of data from
instruments I, II, III and IV (see text).

Data storage is often an intermediate step between acquisition and process-
ing. The so-called 'primary' storage devices used for such a purpose include
the computer's built-in memory and any add-on memory expansions, while 'second-
ary' storage devices usually refer to auxiliary memory modules also known as
'mass memory units' such as magnetic tape or disk drives. Primary devices fea-
ture the advantage of being directly addressable by the computer's CPU. As
they are made of semiconductors and their functioning involves no moving
parts, they have very short access times (of the order of a few nanoseconds)
and are ideal for fast data acquisition. Their capacity varies from computer
to computer and is usually between 8 and 256 kb in the smaller configurations
—the commoner in the laboratory. These storage devices are implemented as RAM
(responsible for the direct storage of data), ROM (read-only memory or firm-
ware, and thus inaccessible by the user), PROM (programmable ROM) and EPROM
(erasable, programable ROM). The communication between the CPU and secondary
storage devices, although permanent, is never direct, so that access times are
much longer.

The scheme in Fig. 2.6, reproduced from Barberá [16], is illustrative of
a data acquisition system. The measured variable generates an electrical sig-
nal at the transducer whose nature and oscillation range is matched to the ADC
via a signal conditioner —usually consisting of one or several operational

amplifiers. Once sampled and digitized, the signal is stored in the computer's memory. Figure 2.7 shows the scheme of a configuration for computerized acquisition of data from electroanalytical systems reported by Price *et al.* [17]. It is of special interest as, although many electroanalytical instruments already have built-in microprocessors, these are meant for instrumental control rather than for data acquisition and treatment. On the other hand, personal computers are rarely fitted to electroanalytical instruments in laboratories. As can be seen, the potentiostat generates a given potential function and applies it to the electrochemical cell, while the microcomputer functions to collect i-V data, treat them and, if required, run typical voltammograms or print the results. The microcomputer used by Price *et al.* is a TRS-80 Model I or III, featuring the 8-bit Z-80 microprocessor and 64 kb of RAM and interfaced to different peripherals. The interface's memory buffer links the computer with the interface bus and controls the acquisition process through dedicated software. The potentiostat output contains two types of information, namely the potential difference between both electrodes and the current intensity generated. The latter is transduced to an intensity-proportional voltage by an i-V converter. Finally, the two signals are digitized by the AD converter and transferred via the bus to the interface buffer, accessed by the computer, which is governed by a BASIC program consisting of a mere six sentences, namely:

| BASIC sentence | Function performed |
|---|---|
| 10 FOR I=1 TO 1024 | Starts loop and states 1024 pairs of data are to be taken |
| 20 OUT 236,16 | Actuates the I/O unit |
| 30 OUT 96,1 | Marks start of conversion by ADCs |
| 40 A(I)=INP(96)*16+INP(97)/16 | Input and storage of potentials |
| 50 B(I)=INP(98)*16+INP(99)/16 | "     "     "     " intensities |
| 60 NEXT I | Closes loop |

The collected data can be subsequently manipulated to provide the most significant results from the voltammogram.

## 2.2.2 Data processing

The data collected or stored by the computer are generally processed or treated prior to delivery. This operation is controlled by means of software, the term used to described the set of mathematical or logic instructions (sentences) input by the user and executed by the computer. It is interesting

that the hardware used in data processing is not and should not be the same in every case. Indeed, there are various categories of computers (micro and mini-computers, mainframes, array processors, etc.) whose features —particularly the price/performance ratio—, are best suited to the task in question (*viz.* the automation of a given technique or instrument).

```
┌─────────────────┐                    ┌─────────────────┐
│     PROCESS     │                    │    COMPUTER     │
└─────────────────┘                    └─────────────────┘
         │                                      ▲
         ▼                                      │
┌─────────────────┐   ┌──────────────┐   ┌─────────────────┐
│   TRANSDUCER    │──▶│  CONDITIONER │──▶│  A/D CONVERTER  │
└─────────────────┘   └──────────────┘   └─────────────────┘
```
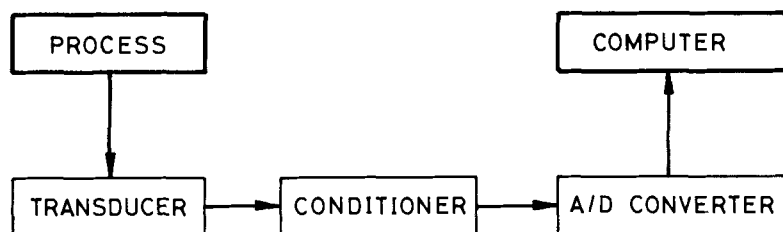
**Fig. 2.6** Block diagram of a data acquisition system.

### 2.2.2.1 Software

Computer software is the tool that actually effects the automation of the data acquisition process. There is a variety of commercial software devoted to this end. Very often, the instruments themselves are furnished with a simple microprocessor aided by a ROM containing the software required for treatment of the data collected by the detector and offer a relatively narrow choice of options illustrated in the instrument catalogue. Other, more sophisticated, instruments are coupled to a more powerful computer and various peripherals (e.g. auxiliary storage devices) intended to run software packages for a variety of purposes (e.g. HPLC experiments) and normally supplied by the same commercial firm. The chief advantage offered by the use of such commercially available software is the need for no programming skills from the operator, who can look up in the catalogue what instructions to key in to obtain the desired results and needs to know no more than the potential and limitations of the measuring instrument and the software controlling it.

Commercial software packages, however, limit in many cases the instrument's scope and flexibility. This is sometimes the result of the software writer following basic guidelines and not having enough chemical knowledge to recognize the subtleties behind the problems being addressed. In addition,

standard software takes no account of the possible changes in the analytical method or technique that the experimenter is sometimes compelled to introduce. In such cases, the operator's knowledge of programming languages (BASIC, FORTRAN) is a great asset as it allows him to adapt available software or write it from scratch for a particular purpose.
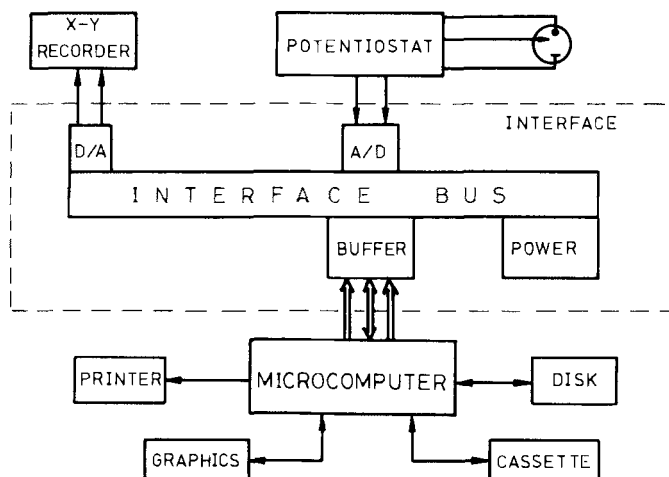


**Fig. 2.7** Scheme of computerized data acquisition from electroanalytical instruments. (Reproduced with permission of the American Chemical Society from ref. [17]).

A piece of software generally consists of two parts. In the first part, *data section*, are defined the variables, data and comments handled by the program. In the second, *algorithm section*, are contained the executable instructions. Prior to writing a program it pays to construct a rough sketch or, even better, a flow diagram clearly describing its structure and the ways along which information (data, instructions, addresses) is to flow before the final stage, delivery of results, is reached. The program can be prepared to process data during the real working time of the instrument (acquisition-processing) or, alternatively, data can be treated only when their acquisition has been completed (acquisition-storage-processing). The former alternative is obviously the more complicated as the time taken to process each piece of data must be shorter than that elapsed between the acquisition of two successive pieces of data.

Essentially, a computer program performs a series of basic functions:

(a) Commanding data input and output of results;

(b) Assigning variables their values;

(c) Carrying out mathematical operations or computations;

(d) Repeating the operations included in a loop; and

(e) Making decisions on the basis of comparisons between data or results.

These operations are enacted by means of instructions written in the so-called 'high-level languages' (BASIC, FORTRAN and PASCAL, principally) and aimed to achieve the desired results.

The different nature of the programs gives rise to *numerical* and *non-numerical* data processing.

**Table 2.1** Applications of numerical processing software in analytical chemistry

| | |
|---|---|
| - Optimization of variables | |
|      Simplex method | |
| - Calculation of parameters | |
|      Thermodynamic | Equilibrium constants |
| | Stoichiometries |
|      Kinetic | Reaction rates |
| | Rate constants |
| | Partial reaction orders |
| | Activation energies |
| - Quantitation of analytes | |
|      Equation fitting | |
|      Characterization of | |
|      calibration graphs | |
|      Resolution of systems | |
|      of equations | |

Numerical data processing involves the manipulation of scalars, vectors or matrices that can be related to curves, surfaces or different equations in general. It is probably the commoner alternative for the treatment of experimental data, which are transformed mathematically by the program. The mathematical transformations involved can be as simple as the characterization of a calibration graph by the least-squares method or as sophisticated as Fourier [18] or Hadamard [19] transforms. Behind these two extremes lie different mathematical models accounting for some physico-chemical phenomena (e.g. the solution to the diffusional-convectional transport equation as descriptive of dispersion in FIA [20]). Insofar as many experimental procedures, particularly

in the field of analytival chemistry, involve quantities and measurements sub-
ject to inherent uncertainty, another major application of numerical process-
ing software is the statistical treatment of data, of great interest in the
detection of errors, their magnitude and contribution to the results. The few
commercially available packages prepared for this purpose should be used with
some caution [21]. In Table 2.1 are compiled the most significant applications
of numerical processing software in analytical laboratories.
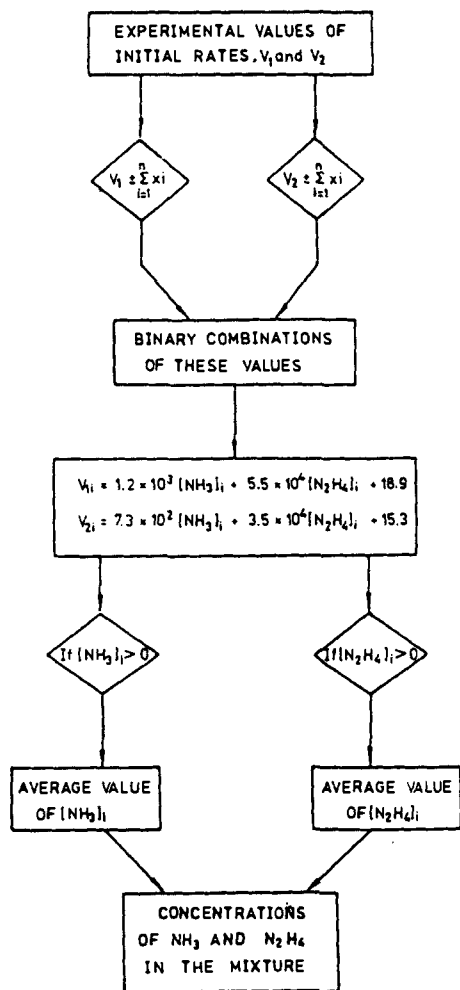


**Fig. 2.8** Flow diagram of the program for the resolution of ammonia-
hydrazine mixtures by the iterative proportional-equation method.

Computerized numerical data processing usually involves two types of er-
ror, namely:

(a) *Rounding errors*, arising from the instrument's limitations in dealing with fractional numbers such as 1/3 (0.33333...) or quantities such as $\pi$ (3.1415926...). Only a number of digits, n, dependent on the number of bits used for data storage, can be retained in such cases. Occasionally, the errors involved can be significant for the final results, although they can be estimated by probability calculations or approximations.

(b) *Truncation errors* are the result of replacing an indefinite mathematical expression with a definite expression. Such is the case with some expressions containing SIN, COS, LGT, SQR or EXP functions.

Figure 2.8 depicts the flow diagram of an off-line numerical data processing program for the resolution of ammonia-hydrazine mixtures by the proportional-equation method, proposed by Ríos *et al.* [22] and of great use in those instances where the two equations arrived at are very similar (one is a linear combination of the other). This makes extremely small variations in the measured experimental parameters (inital reaction rates) result in large errors in the results. Once the experimental data (reaction rates $V_1$ and $V_2$) have been input, the BASIC program establishes two sets of rates, $v_1[V_1 \pm \Sigma x_i]$ and $v_2[V_2 \pm \Sigma x_i]$ by increasing the initial $V_1$ and $V_2$ values at intervals ($\pm x_i$) from $V-nx_i$ to $V+nx_i$. The program tries every possible combination between the two sets and solves the system of equations for each pair. It discards those solutions which fall outside the concentration range where the method is applicable and establishes two new sets of ammonia and hydrazine concentrations. After averaging out both sets of data, the program delivers the final results. The kinetic method, which is not viable in the conventional manner, offers satisfactory results when implemented by this iterative program.

Figure 2.9 illustrates the application of an off-line numerical data processing method, of greater practical interest than that described above on account of the direct acquisition of data from the experimental system by the computer. The method was conceived by Ruz *et al.* for the automation of an FIA system used in the speciation of chromium in water [23]. The configuration is depicted in Fig. 2.9a, and the flow diagram of the program controlling its operation appears in Fig. 2.9b. The FIA system has two detectors, a potentiometric detector providing the pH of the water sample and a photometric detector giving the Cr(III) and Cr(VI) contents sought. From these data and the constants of the equilibria involved, the BASIC program calculates the concentration of the different chromium species (up to nine). The program inputs the data from the detectors, characterizes the calibration graphs run for Cr(III) and Cr(VI) and stores them, indicates whether it is ready to analyse the following sample and does it if requested or stops the operation if told to do so. The microcomputer used is a Hewlett-Packard Model 85, furnished with an HP-IB 82937 interface and an HP 3478A multimeter.
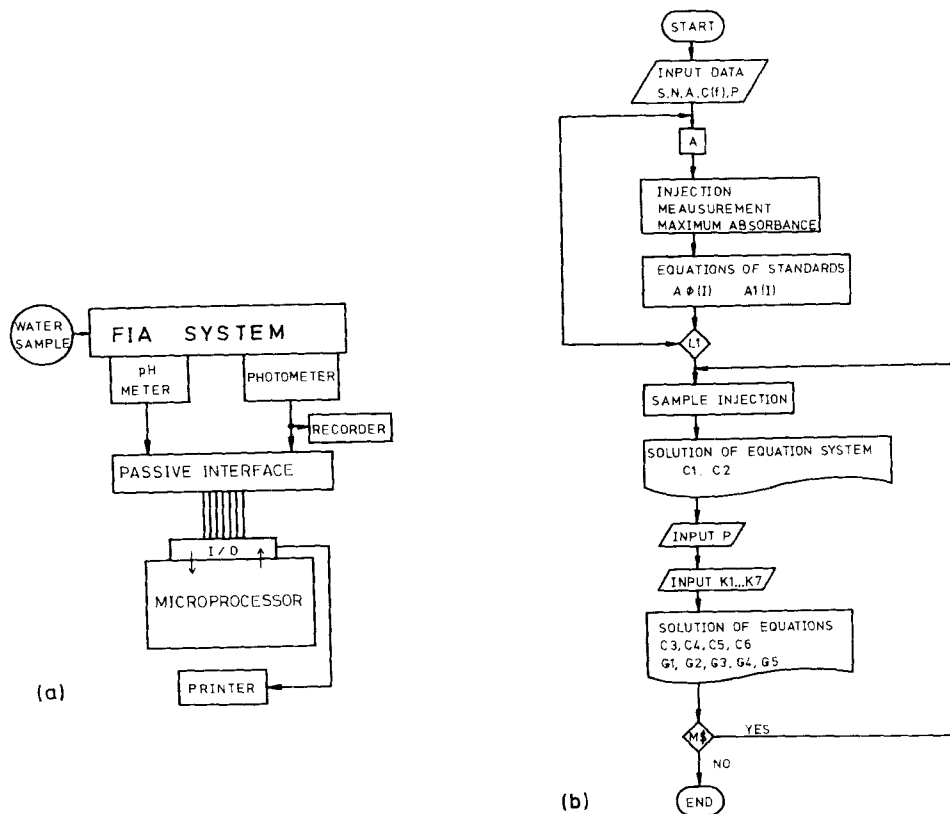
**Fig. 2.9** Speciation of chromium in water. (a) Scheme of the configuration; (b) flow diagram of the program.

As stated above, non-numerical data processing software deals with data that cannot be in terms of numbers (e.g. chemical names and formulae, properties or characteristics of substances). The commonest application of these programs is perhaps the search through the libraries with which some laboratory instruments are provided for spectrum identification (e.g. the characterization of organic compounds by MS, NMR, IR or UV spectroscopy). The operational principle behind the technique is simple: the spectrum of the unknown is compared with those of a series of standard spectra from well-known compounds stored in the computer's memory, which must be large enough to hold a reasonable number of standards. Because of the similarity of some spectra, the computer frequently offers a list of candidates (with their percentage likelihood) rather than making an unequivocal assignment.

Other applications call for more complex data structures capable of storing and handling vast amounts of information such as the properties of a group of similar substances and compiling literature data available on a given technique. In these instances, languages such as PL/1 [24] or PASCAL [25] are to be preferred to BASIC or FORTRAN.

The computer is an ideal tool for the creation (*simulation*) of different models describing the physico-chemical facts underlying a number of processes. Once the model has been constructed from characteristic parameters and its consistency with experimental facts checked, it allows the realization of countless simulated experiments in a short time. This, in turn, allows one to formulate predictions about the real system or even to estimate quantities that cannot be measured experimentally.

Finally, some supercomputers and multiprocessors allow for the so-called *array processing*, namely the simultaneous, parallel handling of various types of information.

### 2.2.3 Delivery of results

This is the last phase of the analytical process and simply provides the user with processed data presented in a comprehensible manner suited to the demands or nature of the analysis concerned. The term 'results' is somewhat ambiguous. Thus, Barker [1] categorized results as:

(a) *Basic results* or values obtained directly from analytical instruments. They correspond to the situation of an instrument's LEDs and readings from analogue or digital instruments.

(b) *Derived results* are obtained upon transformation or processing of basic results and can be numbers, text, graphs or sound signals.

(c) *High-level results*, obtained after ordering, structuring and/or expressing derived results. They are passed on to other users in the form of

aural or written decisions, written paragraphs, papers, publications and theories, and books and theses.

Computers are of particular relevance to the obtainment of derived results, although they can also be used to obtain high-level results (e.g. in word processing).

Results can be delivered by means of a large variety of peripherals fitted to the computer output. The commonest of such peripherals is no doubt the visual display unit (VDU), usually a cathode ray tube (CRT) similar to a TV set, indirectly linked to the keyboard to receive the information typed in. Printers and plotters are probably the most important result display devices with a view to automation as the results, whether numbers, text or graphs, are permanently printed on paper and are recorded after each analysis without the need for the operator's presence.

Appropriate result delivery depends much on the software used for the purpose. The results can be presented as lines, tables and/or graphs. The different programming languages have their corresponding instructions for choosing each type of display. Even graphical presentations, the most complicated from a programming point of view, are currently implemented by various commercially available software packages.

## 2.3 CONTROL OF AN INSTRUMENT OR ANALYSER

The computerized control of instruments and analysers has been common practice in analytical laboratories for several years. Thus, as described in subsequent chapters, the different functions or elements of optical or electrical instruments and analysers can be governed by a microcomputer, which also usually controls the sampling, data processing and result delivery operations. Such control is enacted through *active interfaces* consisting of digital-to-analogue converters (DACs) which actuate mechanical elements by transforming the digital signals from the computer into analogue signals (generally voltages).

The simplest computerized configuration for data acquisition and process control consists of the following elements [26]: the central processing unit (CPU), an EPROM holding the program to be executed, RAM to store data and intermediate variables, and a series of auxiliary components such as a clock and several address decoders. The DACs in the interface play a key role in transforming the signal from the computer output into one transferable to a mechanical element. This requires DACs to preserve the signal with time and to convert it to a proportional voltage level. The circuitry involved in the transformation is composed of a latch ensuring preservation of the computer signal and the converter proper which, aided by an operational amplifier, transforms

it into a voltage whose maximum and minimum can be regulated by means of gain and zero-setting potentiometers (Fig. 2.10).
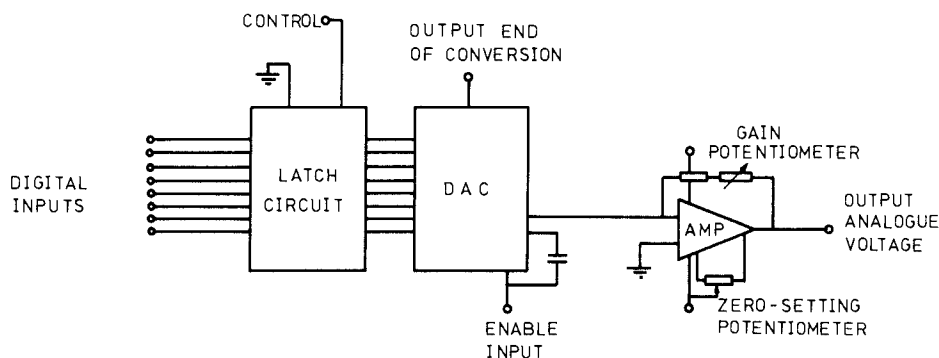


**Fig. 2.10**  Scheme of an active interface furnished with a DAC.

Figure 2.11 illustrates the computerized control of a thermobalance for integral and differential thermal gravimetric analyses [27]. The configuration includes a Nova 2 computer that can be programmed to (a) control the furnace temperature continuously, (b) measure the furnace and sample temperatures continuously, (c) measure the sample weight continuously and (d) command x-y recordings by the plotter. The furnace is taken to the required temperature by a program whose digital information is converted to an analogue voltage actuating a thyristor that controls the furnace power.

Microcomputers can also control one, several or all preliminary analytical stages (sampling, physico-chemical treatment and analytical reaction). In Fig. 2.12 is depicted an FIA autoanalyser for the determination of water pollutants allowing the automation of the sampling and analytical reaction involved by means of the microcomputer used. The water sample to be analysed is circulated as a carrier along the system (Fig. 2.12a) and each pollutant —three altogether— is determined by injection of a selective photometric reagent. Prior to each injection, the water stream is merged with a buffer suited to the analyte to be determined. In addition to the mandatory CPU, the microcomputer used has both RAM and EPROM, and an I/O interface. It usually controls the stop and start of pumps $P_1$ and $P_2$, the switching of selecting valves $S_R$ and $S_B$ in choosing the required reagent and buffer, and the injection of the selected reagent through valve IV.
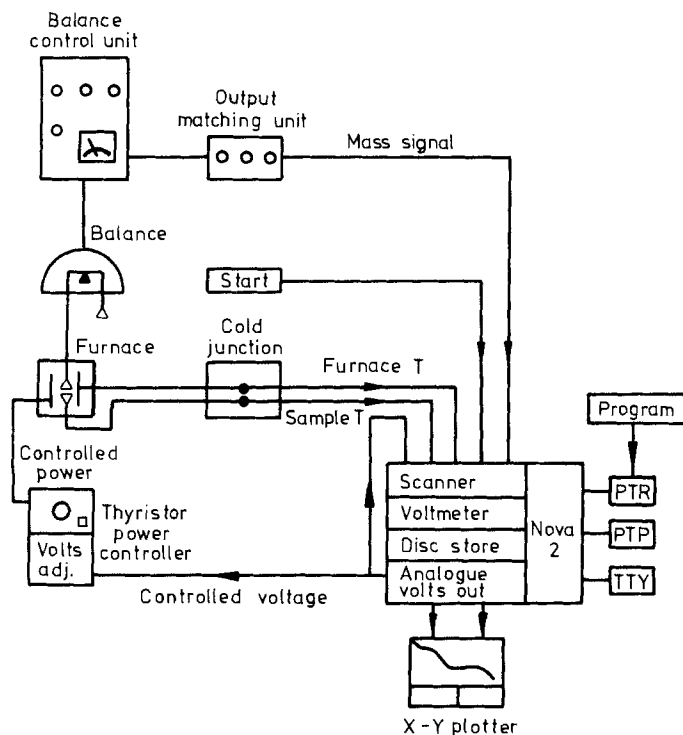
**Fig. 2.11** Computerized control of a thermobalance. (Reproduced from [27] with permission of the Royal Society of Chemistry).

The computer is programmed according to the timing diagram in Fig. 2.12b. Once a calibration graph has been run for each analyte, the determination of the respective concentrations requires programming:

(a) Operation of the pumps: $P_1$ controls the automatic introduction of sample into the system, while $P_2$, aided by valve IV, allows the automation of the analytical reaction.

(b) The time over which each reagent and buffer is injected through valves $S_R$ and $S_B$, respectively.

(c) The time during which the injection valve (IV) remains in the evacuation (reagent injection) position.

Pumps $P_1$ and $P_2$ are started at the beginning of each new analysis (logical state 1). Simultaneously, valves $S_R$ and $S_B$ select the first reagent and buffer lines (analysis for pollutant 1). After a moderate time the injection takes place (IV is switched from logical state 0 to 1). Once this has been completed, IV is switched back to the filling position (logical state 0) and, simultaneously, $S_R$ and $S_B$ select the second reagent and buffer lines (determination of pollutant 2) —$S_R^1$ and $S_B^1$ are switched to logical state 0, while

a)



b)
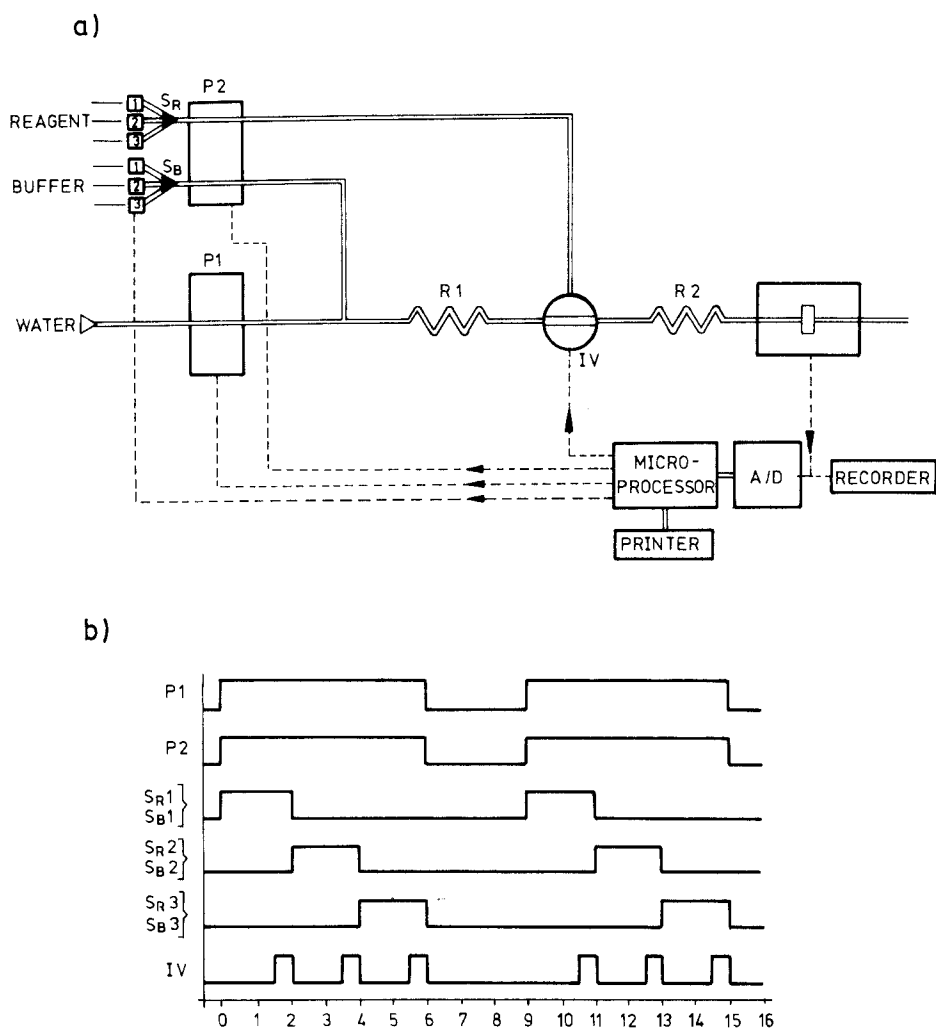


**Fig. 2.12**  FIA Autoanalyser for determination of water pollutants. (a)
Scheme of the configuration; (b) timing of the automatic operation.

$S_R^2$ and $S_B^2$ are switched to logical state 1. The cycle is then repeated as for the determination of the first pollutant. Once the injection of the reagent in line 3 has finished and IV has been switched back to logical state 0, $S_R^3$ and $S_B^3$ are switched to state 0, and so are $P_1$ and $P_2$ (i.e. the pumps are stopped), the anlysis being finished at that moment. The cycle is repeated throughout after pre-programmed intervals, thus allowing the automatic periodic control of the three parameters concerned. As can be seen from Fig. 2.12a, the microcomputer also controls the automatic sampling, data acquisition and result delivery.

Prop *et al.* automated an FIA system including a sampling turntable [29]. The hardware used consisted of a Hewlett-Packard 9845B computer furnished with a real-time clock, a parallel 16-bit HP 98032A interface and timer coupled to the injection unit (BIFOK FIA 05). The data acquisition operation is automated by means of the parallel 3-digit (0-1 V) BCD output of the photometer, a BIFOK FIA 06. The role of the turntable (Skalar Sampler 1000) is similar to that played by selecting valves $S_R$ and $S_B$ in the configuration described above. However, the analytical chemical principles behind the two approaches are different: this configuration is intended for the determination of a single parameter in a large number of samples, held in the turntable cups, while the configuration above was conceived to determine three parameters in a few samples. The software used by Prop *et al.* is written in BASIC and consists of ten subroutines and functions which control the operation of the turntable, injection unit and photometer via the computer's interface.
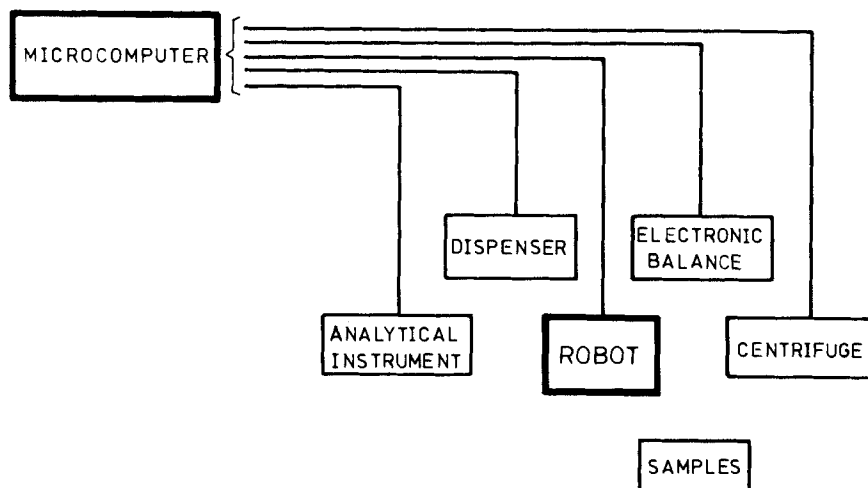


**Fig. 2.13** Scheme of an automatic robot station. (Reproduced from [30] with permission of Elsevier).

The laborious, complex treatment occasionally required by some samples is begining to be affordable for automation thanks to the use of robots in the laboratory. These restlessly mimic the actions of operators, for whom they make ideal stand-ins. The control of a robot station, the operation of which is described in detail in Chapter 9, would be virtually impossible without the aid of a computer directing the robot's movements and dealing with the measuring instruments involved. Figure 2.13 shows the scheme of a generic robot station [30] and emphasizes the significance of the role played by the computer which, in addition to directing the robot —situated in a strategic position for easy acces to all the units in the station—, collects data and controls the remainder of the units (balance, centrifuge, dispenser and analytical instrument).
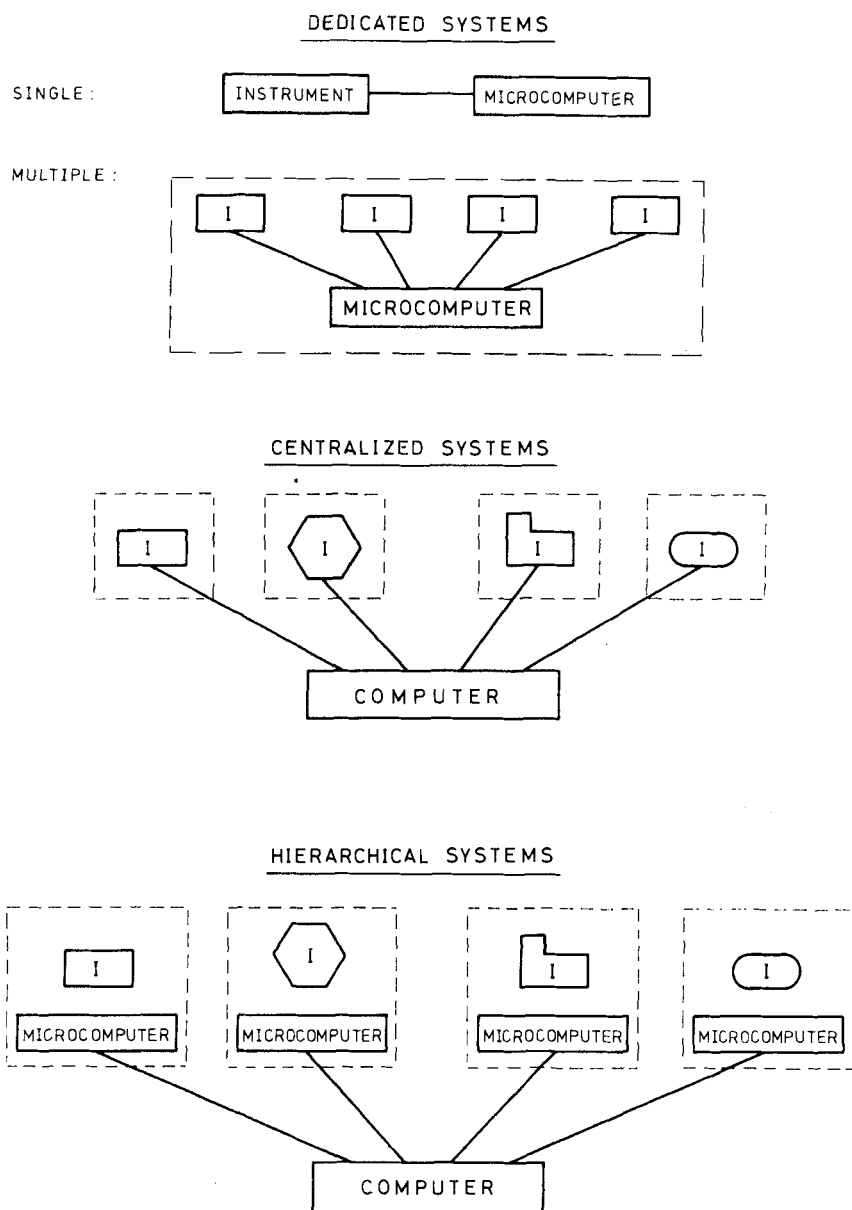
## 2.4 COMPUTERIZED CONFIGURATIONS

Today's laboratories —particularly the larger ones— use a variety of intelligent, microprocessor-controlled, instruments with analogue output and (micro)computers interfaced to one another. It is the fashion in which the interfacing is done that ensures efficient laboratory computerization (automation). Ziegler [31] established three categories of computerized configurations, namely (Fig. 2.14):

(a) *Dedicated systems.* In these, the computer is exclusively devoted to  a given analytical task. They can be 'simple' or 'multiple' depending on whether each instrument is controlled by a different computer or the same computer governs more than one instrument of the same nature. The former are simpler and commoner, and the latter are of use when the instruments are all located in the same vicinity, the sample throughput required is not very high and flexible software allowing simultaneous operations to be controlled through appropriate interfaces is available. Multiple dedicated systems are frequently used in industrial or control laboratories (e.g. in controlling several gas chromatographs). They can pose problems arising from a lack of coordination or from a limited memory storage capacity.

(b) *Centralized systems,* in which a powerful computer is interfaced to a series of instruments of the same or different nature. They have lost popularity on account of the falling prices or microcomputers, the problems encountered in high-speed data acquisition and their cumbersome operation.

(c) *Hierarchical systems,* where each instrument or analyser has a dedicated built-in microprocessor which solves specific problems confronting the particular analytical technique applied. Such microprocessors are not prepared to deal with and store large amounts of data, so they are connected to a cen-

DEDICATED SYSTEMS

SINGLE: INSTRUMENT —— MICROCOMPUTER

MULTIPLE:

```
   I        I        I             I

            MICROCOMPUTER
```

CENTRALIZED SYSTEMS

```
  I        I         I            I

              COMPUTER
```

HIERARCHICAL SYSTEMS

```
     I          I            I              I

 MICROCOMPUTER  MICROCOMPUTER  MICROCOMPUTER  MICROCOMPUTER

                    COMPUTER
```

**Fig. 2.14** Configurations for laboratory computerization: dedicated (simple and multiple), centralized and hierarchical systems. I: instrument.

tral computer which undertakes these functions. This type of system combines the advantages of dedicated and centralized systems and overcomes the problems posed by both.

### 2.4.1 Workstations

On the basis of hierarchical systems, some companies such as Perkin-Elmer and IBM have developed the so-called 'workstations'. These are interactive computers based on instruments that allow the user to control one or most of the laboratory operations. Hence, they afford data acquisition, classification, amendment, correlation and request, and support data processing and/or process simulation.
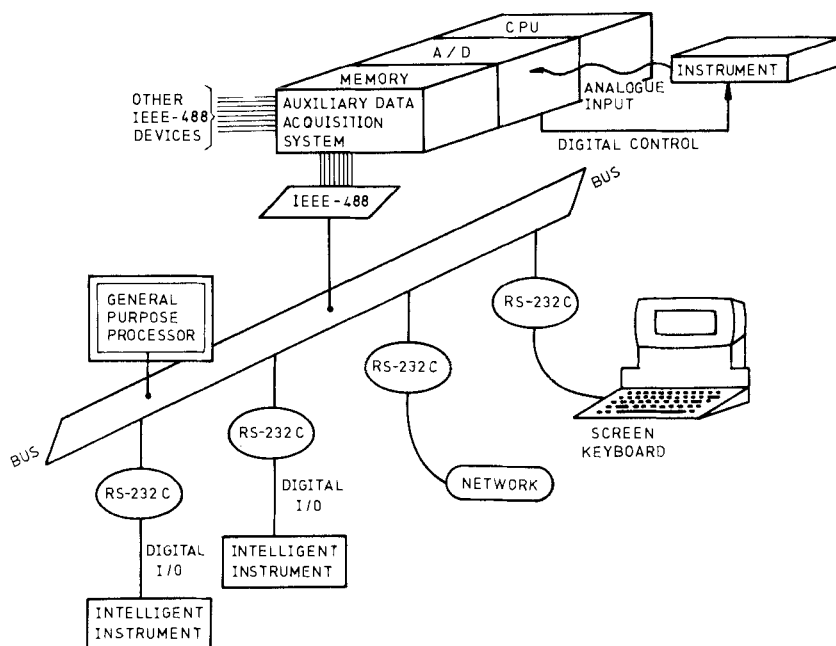


**Fig. 2.15** Scheme of a workstation (Professional Perkin-Elmer Series 7000 computer). (Reproduced from [32] with permission of the American Chemical Society).

Figure 2.15 depicts a workstation for laboratory applications in general. It is a fairly simple, professional Perkin-Elmer PE-7000s configuration [32] with the following features:

(a) The station's brain is a 16/32-bit Motorola 68000 microprocessor with expandable addressing and prepared to run both BASIC and FORTRAN software. The

ROM and RAM, massive-storage hard-disk drives, a standard RS-232C interface op-
erating serially at 9600 Baud (bit/s) and a parallel one transmitting at 100
Baud make up this section of the system.

(b) As far as  performance is concerned, a generic  station such as this
can  be readily adapted for control and acquisition of information from vari-
ous intelligent instruments —provided with a digital output— such as IR,
UV-visible and atomic absorption spectrophotometers, fluorimeters, chromato-
graphs and thermal analysers. Such instruments can be directly linked to the
workstation via the ports of the RS-232C interfaces and be prepared for a com-
plete analysis. The software used is stored in a Winchester disk drive. For
instruments lacking digital output, the station has a special data-acquisition
module linked to the IEEE-488 interface and consisting of an ADC controlled by
a dedicated microprocessor (CPU). It is capable of storing up to 1000 values
in RAM for subsequent reading.

(c) Regarding external communication, the station was conceived to be com-
patible with Perkin-Elmer computers of the 3200 series, thereby allowing mu-
tual communication between terminals.

## 2.5 EXPERT SYSTEMS

While the success of computers in most fields of application lies in their
capability to accelerate processes, decrease costs and save human labour, they
machines should be understood rather as intelligence amplifiers in the scien-
tific field; hence the current allusion to *artificial intelligence* (AI) to
refer to the science of computer-acquired knowledge. Indeed, AI is the re-
sponse to the need for software mimicking man's thinking activities, i.e. pre-
pared to deal with the representation, manipulation and construction of know-
ledge about facts, actions and random laws.

This type of software converts computers into *expert systems* capable of
solving difficult problems calling for specific experience in the particular
field of application [33]. Such software is rarely written in high-level lan-
guages such as BASIC, FORTRAN or ALGOL, but is rather made of fixed structures
with pre-sequentialized sets of precise instructions capable of 'reasoning' by
themselves or, in other words, of choosing the correct solution from a number
of solutions. This artificial reasoning is made through symbols contained in
programs usually written in LISP [34] or PROLOG [34,35].

As far as the area of knowledge is concerned, AI software should be de-
veloped by first defining the knowledge domain of the particular problem ad-
dressed. Thus, the domain of the program DENDRAL is the structural elucidation
of organic compounds, while that of the program MYCIN is the medical diagnosis

of bacterial diseases. These programs work on a data or basic knowledge base containing all the information available on already known systems and the logical bases for their reasoning, so that they manipulate the available knowledge until they find a final solution.

Knowledge manipulation, as important as the definition of the knowledge domain, is implemented by a *rule interpreter* consisting of three parts, namely a *pattern matcher* prompting the rules to be applied in each situation, a filter determining which rule of several possible is to be applied and an *executor* eventually applying the rule.

As the programs used by expert systems are long and complex, it is difficult to coordinate new knowledge or rules with those already present or to modify them. In this respect, there are few programs that allow the incorporation of new rules without altering the structure of the main program. Alternatively, a dedicated program can be written for such a purpose (e.g. meta-DENDRAL, designed to expand or amend the original DENDRAL).

Expert systems usually work by first establishing a dialogue with the user via consultation programs. The system requests information about the problem. If the user does not fully understand the question, the system provides a comprehensible explanation. Once the problem has been solved, the system indicates the reliability of the solution given. As such a solution is merely a hypothesis formulated to account for the experimental facts observed, the user will accept it only if it is consistent —particularly if a different solution was expected.

Expert systems have been used in chemical laboratories for a host of applications such as the design of synthetic pathways, structural elucidation of organic compounds, interpretation of IR spectra, determination of the composition of rocks by X-ray analysis and analysis for active principles in drugs. This range of applications will foreseeably be expanded by addressing many other problems currently confronting chemists. However, as pointed out by Kateman [36], these systems should be accepted as "an interesting symbiosis between artificial and human intelligence to arrive at the optimum solutions of a problem", but never to the point of completely replacing the specialist.

### 2.6 COMPUTERIZED ACCESS TO SCIENTIFIC INFORMATION

Information has no doubt become a strategic resource in every conceivable area. The scientist has always felt the need to be well informed about new knowledge, advances and research. However, the amount of information available is so vast that the scientist would be overwhelmed if such information were not preserved and organized in the efficient way only computers can afford.

Large libraries have been replaced by small massive digital memory storage units governed by a central computer linked to a vast terminal network. These are the so-called 'data banks' or 'data bases', a homogeneous collection of information items stored on magnetic media and accessible by computers. They differ from other information media in three essential aspects, namely:

(a) The information base is made of a set of computer elements with a homogeneous structure (papers, literature references) and similar contents.

(b) Consultation is made with the aid of a computer and the set of instructions channelling the user's requests.

(c) Information is transmitted through a network —usually the telephone network—, so tat the terminal is always to hand.

The use of data banks usually involves a chain of five links, namely:

(1) *Editors*, who collect information, organize it and record it on magnetic media.

(2) *Dealers* are supplied with the recorded magnetic media and manage them in their host computers to make the registered information available to the end users.

(3) The *network* links the users to the host computer. A few international companies serve this purpose throughout the world. An example is the USA-based intercontinental INFONET network, which links computers via coaxial leads and artificial satellites. The telephone line is also commonly used as the vehicle for transmission of scientific information.

(4) *Information brokers* are professionals performing specialized searches for their clients, relieving them of the burden of treading on poorly known ground.

(5) *End users*, the final target of the process are connected to the host computer, from which they receive the requested data via a visual display, a printer or both.

In addition to this well-organized access to stored information, computer users have the choice of building laboratory-scaled, home-made data banks or files to organize the information of interest available on a narrower area of knowledge or a given research line. The literature or information required is compiled, classified, labelled, coded and finally stored in some massive memory storage unit. If the information is labelled according to titles or keywords, retrieval by searching for the prompted concept is normally fast and straightforward.

## 2.7 FINAL REMARKS

From the argument above one may conclude that computers are a boon insofar

as they relieve laboratory workers from a host of routine tasks that can be automated with their aid. Despite their advantages, computers used in laboratory automation should meet a series of requirements to be fully acceptable for such a purpose, namely:

(a) They should not restrict the instrument's capabilities (e.g. spectral resolution, time-dependent resolution, dynamic signal range, sensitivity). In fact, problems occasionally arise from the speed of data acquisition (dependent on the nature of the ADC used) or the broad dynamic signal ranges to which the ADC's digital sampling resolution is not matched.

(b) They should be modifiable and expandable, i.e. the user should be capable of modifying the software available or developing it himself. This requires data-acquisition and data-processing software to be available in independent packages. Usually the former is written in machine code, which affords higher sampling frequencies, whereas the latter is written in some high-level language such as BASIC or FORTRAN. On the other hand, the hardware should be expandable to serve further purposes.

(c) The communication between the instrument and the analyst via the computer should be convenient and reliable in order to prevent misunderstandings and avoid or minimize errors, which should be detected and corrected as far as possible. The operator should be capable of going through any part of the process and modifying it as required (i.e. the system should be automatic, but also flexible).

(d) The system should have a reasonable amount of memory determined by the particular tasks undertaken. Thus, pattern recognition, which involves holding and handling a vast collection of NMR, IR and mass spectra, calls for large memory buffers.

(e) It should be linkable to other systems for exchange of information via the hardware and the software. This is difficult because of the incompatibilities between the hardware developed by different companies, which can be overcome in some cases, however, by the so-called 'hardware links'.

Another major consideration on the use of computers in the laboratory is the way in which the problem concerned is addressed. The replacement of manual labour with computerized control can be

(a) *Convenient*, i.e. the problem addressed does not specifically require replacing any of the stages in the process, but doing so certainly saves labour and reduces the risk of errors.

(b) *Necessary*, i.e. the analytical process is perfectly viable without the aid of a computer, but some aspects of the problem involved (e.g. a large number of samples, the need for a high throughput) demand automation and hence the use of a computer.

(c) *Indispensable*, when the analytical technique cannot be applied without the aid of a computer. Such is the case when:

(1) A large number of samples need to be processed and several parameters determined rapidly and accurately (e.g. in clinical laboratories). In fact, both continuous (SMAC) and batch (RA 1000) analysers are fully computer-controlled.

(2) A high speed of data acquisition is required (e.g. in the stopped-flow technique or with diode array detectors).

(3) A large number of data are to be processed, as is the case in Fourier transform IR spectroscopy.

(4) The technique used involves extremely long times (e.g. single-crystal X-ray diffractometry).

Current trends in computer science seem to point to the construction of fifth-generation computers. There is a revolutionary and ambitious project by the Japanese intended to develop computers capable of processing knowledge rather than data. Among the essential functions of these new machines would be those of learning, associating concepts, inferring consequences, making judgements, holding a question-answer dialogue with the user, solving problems addressed as a whole and using in an intelligent manner the information stored in the data base by grasping its meaning rather than merely storing, retrieving and delivering it.

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# 3
## Automation of sampling

### 3.1 INTRODUCTION

As stated in Chapter 1, the first few stages of the analytical process are also the more complex and the source of potential major errors, and the concept of a 'sample' is rather extensive.

The automation of preliminary operations in the analytical process is rendered particularly difficult by (a) the large variety of existing samples, available in all three states of aggregation (solid, liquid and gas) and in different particle sizes, (b) the diversity of circumstances (sampling location and distance to the laboratory, need for preservation) and (c) the pretreatment required (dissolution, preconcentration, interference removal, etc.). All this makes the first stage of the analytical process one that cannot be automated in every case; in fact, endeavours in this field are often aimed at a particular type of sample or application (e.g. clinical, food, agricultural or pharmaceutical analysis).

Preliminary operations usually consist of a number of steps, all of which can in principle be carried out without human intervention. For simplicity, the target of automation can be sampling, sample treatment or both sub-stages. The last situation is by far the commonest, even though the complete automation of the entire sampling stage is difficult.

Automatic sampling and treatment systems (ASP or automated sample preparation according to Burns [1]) can be incorporated into the analytical process in one of three fashions, namely:

(a) As one of the dedicated elements or modules of an automatic analyser. They are the key elements in discrete analysers. Continuous analysers, on the other hand, use a module containing the sample set to be analysed (sampler) and perform the required treatment in the flow system. In robot stations, various accessories (sample tube racks, exchangeable pipette tips, dilutors, extractors, stirrers, etc.) and instruments (electronic balance) carry out the different preliminary operations.

(b) By on-line adaptation of a module to an analytical instrument. Many commercially available instruments such as spectrophotometers and chromato-

graphs can be provided with accessory systems for automatic sampling and treatment.

(c) As a configuration working off-line with the instrument and performing a specific operation prior to introduction, whether automatic or not, of the samples. Automatic dilutors, recently very popular in clinical laboratories, are representative examples of this option.

Preliminary operations have an undeniable significance to the analytical process. They are usually slow and hence decisively influence the analyser throughput. Their difficult complete automation requires human participation to different extents and involves increased analytical costs. In addition, they are a major source of errors —often minimized or neglected, but always as large as or even more significant than those made in other stages of the analytical process—, whether small (e.g. those resulting from incomplete dissolution or extraction) or large (e.g. those arising from a poorly representative sample or the incomplete cancellation of matrix effects).

A serious shortcoming involved in the automation of the first stage in the analytical process is the potential lack of connection between the operator and the operation which, because of its inherent complexity, demands stricter surveillance than other stages and compels the operator to perform periodic tests on standard samples in order to ensure the correctness of the different parameters measured during the different sub-stages. This involves controlling the accurate measurement of weights and/or volumes, dissolution completeness, the efficiency of preconcentration and interference removal operations, and also the correct matching of results to samples and of the different data of each analyte to each sample.

Although this chapter deals with automatic sampling systems separately, automatic sample-treatment systems, the subject of the next chapter, are usually integrated with the former in a single module, so that the distinction is often purely artificial. Occasionally, though, both systems are separate units, the treatment module being included where the analytical reaction takes place and the signal is monitored. Insofar as the different stages of the analytical process are not clearly distinct and depend on the type of sample concerned and the instrument used to measure the analytical signal, some specific systems are also described in other chapters.


## 3.2 FUNDAMENTALS OF SAMPLING

The term 'sampling' is vague [2] as it is used to describe a variety of operations such as (a) collection of the sample from its source (e.g. a patient, lake, waggon or the environment of an industrial area), (b) preserva-

tion of the raw sample collected if the remainder of analytical operations are not carried out in a continuous fashion, (c) reduction of particle size to the extent required by the analytical system available, (d) accurate measurement of the portion to be used for the analytical calculations and (e) introduction of the sample into the analytical processor.

The principal aim of this preliminary operation is to obtain a sample aliquot representative of the material to be analysed.

It is immediately apparent that the complete automation of this sub-stage is a difficult task. Only  in a few instances (e.g. the automatic *in vivo* determinations described in Chapter 14 and performed with the on-line process analysers dealt with in Chapter 17) is this ideal objective affordable. Much more often, some of the above-mentioned operations involve human participation, although it is still termed 'automated' [1]. Therefore, although many clinical analysers are classed as automatic, the blood and urine samples that they handle are collected and even treated manually before they are placed on the sampler. Such is also the case with automatic off-line water pollutant analysers, also calling for manual collection and preservation of samples. Consequently, the 'automated sampling' concept as used here refers to the introduction into the analyser or instrument concerned of a definite portion of sample collected from its source and even treated manually, with the few exceptions stated above.

Automatic sampling systems can be classified according to different criteria (see Table 3.1), namely:

(a) *According to the state of aggregation of the sample.* A distinction can be made between solid, liquid and gas sampling systems, in addition to those specially designed for handling mixed samples (e.g. emulsions, suspensions or liquids containing solid materials). Obviously the foundation and design of these systems will be as varied as the nature of the samples handled can be. This criterion will be used as the basis for a description of the different sampling systems commented on above.

(b) *According to the manner in which the prior transfer of sample is effected.* In continuous automatic sampling, a flowing liquid or gas carries the sample from the sampling spot to the analyser. Such is the case with reverse FIA (Chapter 6) and completely continuous configurations (Chapter 7) or  on-line process analysers (Chapter 16). More often, though, samples are held in independent containers prior to introduction into the analyser or instrument, i.e. sampling is discrete as in both batch, continuous and robotic systems.

(c) *According to  whether or not the sampling also involves  quantitation.* Usually, the sample weight (or volume, if fluid) is measured prior to introduction into the analyser. However, the completely continuous systems referred

to above and pharmaceutical tablet samplers, among others, require no prior sample quantitation.

**TABLE 3.1**  Classification of automatic sampling systems

| | |
|---|---|
| According to the state of aggregation of the sample | Liquid<br>Gas<br>Solid |
| According to the manner in which the sample is transported | Continuously (*in situ*)<br>Discretely |
| According to whether or not the sample is quantized | With weight/volume measurement<br>Without quantitation |

## 3.3 SOLID SAMPLING

This represents the most complex situation in the automation of preliminary operations in general, and of sampling in particular. The chief difficulties involved arise from three aspects, namely:

(1) The sample should be representative of the material to be analysed. Sample collection is, except in a few instances (e.g. in continuous industrial production assemblies, which afford automation), normally carried out manually. Once collected, the particle size should be made homogeneous in order to ensure the presence of all the original ingredients in the final sample. In dealing with compact materials, differences in composition according to sampling zone or depth should be taken into account. All these requirements make human participation virtually indispensable in most cases.

(2) Sample quantitation is a difficult operation to automate. Weighing is in fact an off-line operation that cannot be readily implemented by analyser modules with the exception of robot stations. On the other hand, electronic balances do allow the measured weight to be passed on to the analyser or instrument microprocessor. In this case, the operation is essentially manual as only data transfer is automated. The quantitation of solid samples can be based on:

(a.1) Weighing of a preselected amount. This requires special caution not to exceed the prefixed limit or to remove the excess if incurred. The slowness and difficulty involved in its automation is outweighed by the constancy of the initial data, on which all results are based.

(a.2) Weighing of the amount loaded on to the balance scale. This is the commonest alternative as it merely requires the actual amount weighed each time to be retained for computation of the results and therefore lend itself readily to automation.

(b) Weighing of prefabricated samples which, *a priori*, should be the same weight and in which the analyte concentration must be known in *absolute* rather than in relative terms (e.g. active components in pharmaceuticals). The weighing is therefore dispensable and makes the automation of the operation more affordable.
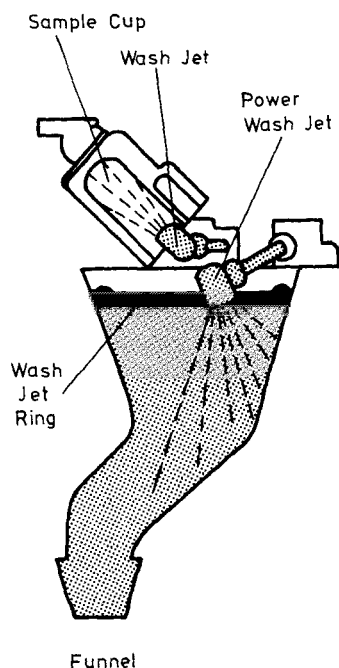


**Fig. 3.1** Addition of a solid sample held in a cup of the SOLIDprep II sampler (Technicon), to the solution vessel through a funnel. (Reproduced with permission of Technicon).

(3) In contrast to the two operations described above, the *incorporation* of the solid sample into the analyser or instrument is comparatively easy to automate. Samplers with cups or vials holding each sample separately are relatively inexpensive. In batch analysers, samples are treated and transferred separately; continuous analysers, which are much commoner, involve intermediate operations (dissolution, extraction, etc.) and do not have many automatic systems available for incorporation of solid samples. One such system is the

SOLIDprep module marketed by Technicon for their AutoAnalyzers (Chapter 5). It consists of a circular sample turntable supporting 20 plastic or glass cups, each of which can hold between 5 and 10 g of the material, depending on its density. When the system is started, the contents of each cup are poured into a common homogenizing container. The solid sweeping system (Fig. 3.1), unnecessary when working with pharmaceutical tablets, involves the use of two pressurized liquid jets, one aimed at the inside of the cup and the other to the funnel leading the suspended sample to the homogenizer. The elements actually coming into contact with the samples are made of inert materials (glass, Teflon, Kel-F, ceramics). Once in the homogenizing container, the sample is added a suitable solvent and is subjected to vigorous stirring at a controlled temperature. Once the dissolution or extraction operation has finished, a withdrawing tip like those typically used by AutoAnalyzers takes an aliquot of supernatant and introduces it into the continuous segmented configuration. A washing system allows each treated sample to be flushed from the system before the next solid sample is introduced.

Robot stations (Chapter 9) are the best alternative to the automation of analytical processes involving solid samples. This is also probably their major field of application as they allow the automation of all the sub-stages involved in the preliminary operations, many of which are unaffordable with most analysers.

### 3.4 LIQUID SAMPLING

Liquid samples are by far the most frequently involved in automated analytical processes, which is not surprising taking into account the few technical difficulties which they pose.

Although liquid samples do pose some problems arising from the nature of the whole material, these are easy to solve. Thus, even low sample volumes can be homogenized in an automatic fashion. Large samples are normally handled by taking different representative samples from a given location. Liquids in motion make sampling essentially time-dependent.

Sample volumes can be measured in any of three general ways: (a) by means of a probe aspirating the liquid at a constant rate, (b) by using mechanically or hydraulically actuated, high-precision syringes for a preset time and (c) by weighing the liquid.

Automatic samplers, which are fairly commonplace in liquid sampling, consist of the following elements:

(a) A sample tray, circular, square or snake-like in shape and  containing the vials or cups intended to hold the non-quantized liquid samples previously

transferred from the source matrix (e.g. a patient, lake, reservoir). Obvious-
ly, the samples must be perfectly numbered and exactly positioned in order to
ensure their accurate identification.

(b) A mechanical device turning the sample tray through the angle required
to place each sample in turn in the aspiration position.

(c) A moving articulated needle fitted to a withdrawal system (generally
a peristaltic pum or a syringe) serving the dual purpose of measuring the sam-
ple volume and introducing it into the analyser. Special credit should be
given here to the withdrawal/pipetting systems made by Hamilton, reknowned for
their accuracy and reliability in measuring micro volumes.

(d) A mechanical-electronic system synchronizing the functioning of the
previous elements. Full synchronization requires programming the times of
sample aspiration, which determines the volume taken, sample changeover and
aspiration of washing solution, or air aspiration in continuous segmented sys-
tems.

(e) Other auxiliary elements such as stirrers and heaters.

Obviously, no sampler is required when sampling is performed in a com-
pletely continuous fashion.

In describing some of the systems available for the sequential introduc-
tion of samples into analysers and instruments, a distinction will be made
according to whether they are used with continuous or batch configurations.
The introduction of liquid samples into robotic analysers shares some of the
features of the operation performed with batch analysers and is described in
detail in Chapter 9.

### 3.4.1 Liquid sampling in continuous analysers

The manner in which liquid samples are introduced into a continuous ana-
lyser depends on its nature. Figure 3.2 illustrates some of the commoner situa-
tions in this respect, which are also commented on below.

In completely continuous analysers, samples are taking from an evolving
system such as a pipe pouring out waste water or an industrial effluent. A
peristaltic pump continuously aspirates the sample, in which the evolution of
one or several analytes is monitored as a function of time. This is the sim-
plest possible alternative and is characterized by the absence of discontinu-
ities; however, the system can be programmed to operate in a discrete fashion
if required. Reverse FIA and completely continuous assemblies (Fig. 3.2a) are
representative examples.

Normal flow-injection analysis (FIA) involves using a peristaltic pump to
aspirate the sample into the loop of an injection valve, which also quantizes
the aspirated volume, and subsequently inserting the loop contents into a car-
rier or reagent stream.
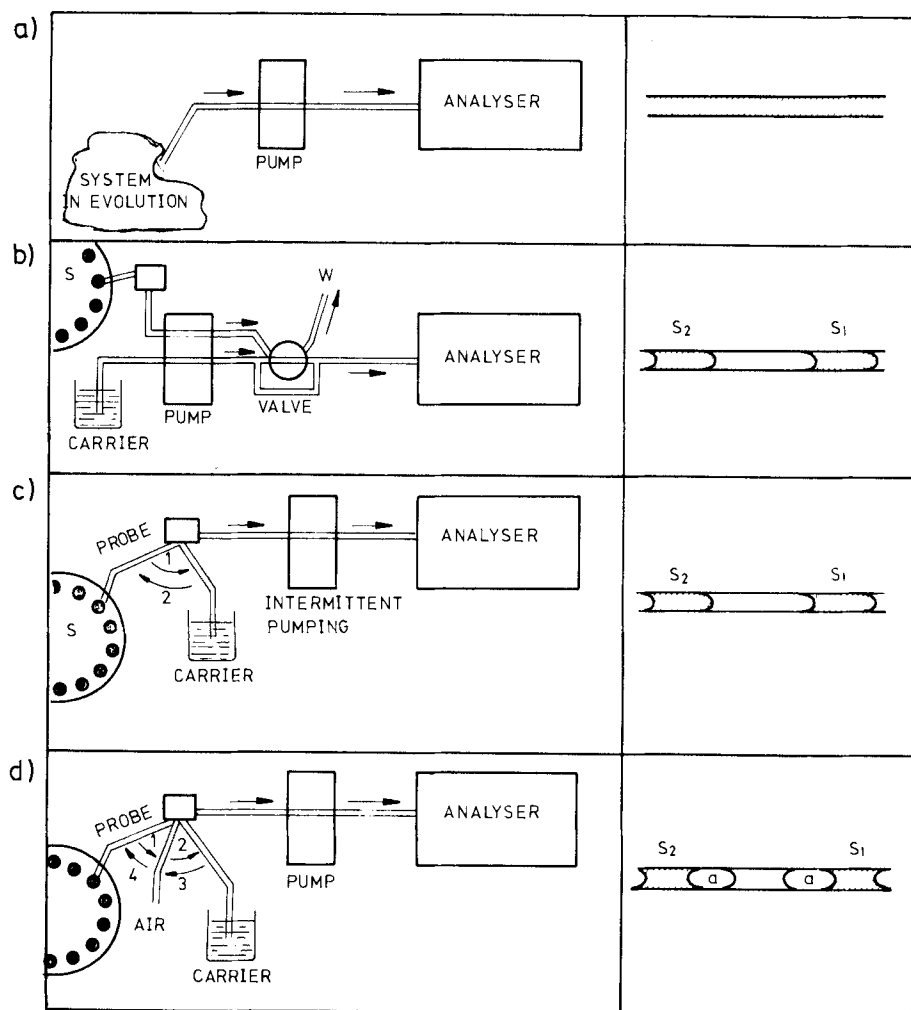
**Fig. 3.2** Different ways of introducing liquid samples into continuous analysers: (a) continuously; (b) by injection; (c) by aspiration without air; (d) by aspiration with air.

The discrete aspiration of a fixed sample volume, determined by the time during which the sample is withdrawn from the sampler and by the flow-rate of the peristaltic pump, can be accomplished in two manners. In the assembly developed by Rocks and co-workers [3,4], the moving articulated needle features two aspiration positions (see Fig. 3.2c). In the first, the sample is aspirated from the sampler vial. Once the programmed volume has been taken, the

peristaltic pump is stopped while the aspirating tip remains in position. Then, the latter is raised and immersed in a carrier or reagent reservoir, after which the pump is re-started. In this manner, a sample plug is inserted into the carrier stream without the need for a rotary valve, whose function is replaced by the intermittent operation of the pump. As no air is introduced into the system, this can be considered an FIA mode. In segmented-flow analysers, the pump works in a continuous fashion and the aspirating tip has three positions in which it aspirates sample, air and washing solution, respectively (Fig. 3.2d).

To the right of Fig. 3.2 is shown the flow profile obtained after the system has sequentially taken two samples, $S_1$ and $S_2$ —except if sampling is carried out continuously. Cross-contamination between samples in the above-described alternatives is discussed in the corresponding chapters.

### 3.4.2 Liquid sampling in batch analysers

The aspiration systems used by batch analysers, normally moving articulated probes, withdraw the samples from the sampler vials and dispense them to the analyser cups or cuvettes. Time is not such a decisive factor here, so that high-precision syringes are the commonest option for measuring and transferring the liquid samples.
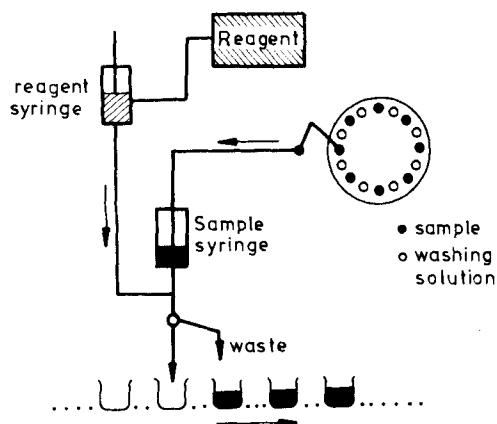


**Fig. 3.3** Introduction of liquid samples into a batch analyser by means of a syringe. There us a further syringe connected to a reagent reservoir. The probe can be switched between two positions for unloading into a cup or to waste depending on whether the syringe is filled with sample or washing solution.

Figure 3.3 depicts a single-syringe sampling system for introduction of an

accurately measured sample volume into a batch analyser. The sample tray holds alternate washing solutions intended to flush assayed samples in order to avoid carryover. One of the positions of the sample probe is a waste port. A further syringe is used to add reagent through the same conduit. This operation is carried out immediately after the sample has been added, which facilitates its flushing.

The transfer of liquid samples by means of a moving articulated needle fitted to a syringe and involving an intermediate washing stage is commonplace in this context. The scheme in Fig. 3.4 corresponds to a configuration featuring a probe with four positions where it aspirates one of the samples held in the sampler vials, dispenses the volume taken to the analyser cup, is submerged in a washing solution reservoir and dispenses the aspirated solution to the waste reservoir. Once the first sample has been processed, the sampler is turned to place a fresh sample in position before a new cycle is started.
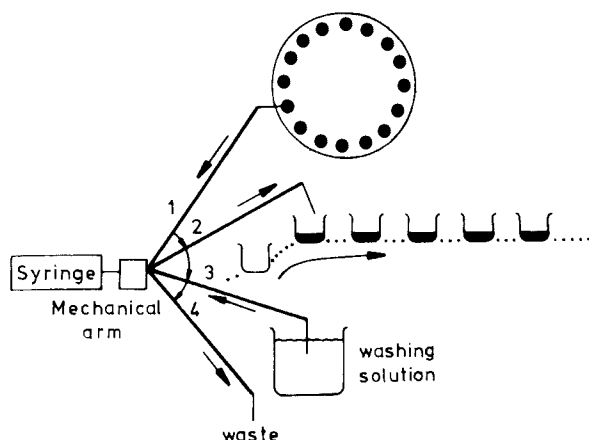


**Fig. 3.4** Transfer of liquid samples with the aid of a moving articulated probe with four positions: (1) aspiration of an accurately measured sample volume, (2) unloading into a cup of the batch analyser, (3) aspiration of washing solution and (4) unloading to waste.

Figure 3.5 depicts the scheme of another alternative to the automatic introduction of liquid samples. It is a non-rotary injection system resembling the injection valves used in liquid chromatographs, although simpler than these as they do not have to withstand such high pressures. In the filling position, the sample is aspirated —by means of a peristaltic pump, for example— into a fixed-volume loop as a syringe is filled with reagent or flushing solution. In the injection position, the system connections are changed

and the syringe propels the carrier solution, which sweeps the sample held in the loop to the analyser cup. In the course of this operation, the sampler turns and a fresh sample is aspirated into the system, so that the first aspirated portions sweep the residues of the preceding sample.
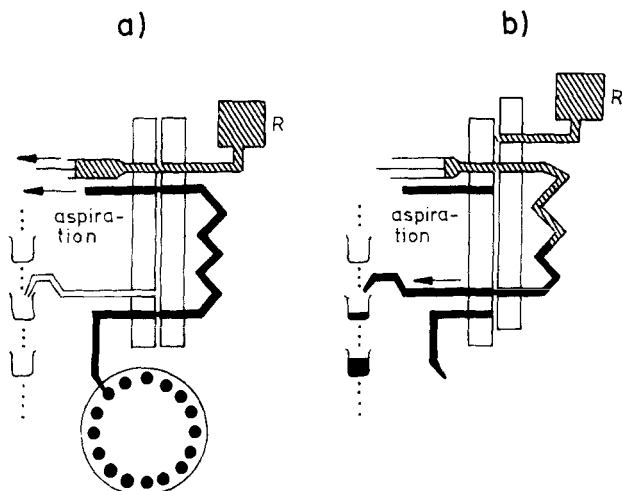


**Fig. 3.5** Injection valve for insertion of a fixed sample volume into a batch analyser. (a) Filling position; (b) evacuation position.

A recently developed sample transfer mode is the Technicon Model RA1000 (Fig. 3.6). It functions similarly to a pipette or an articulated needle, though it lacks the two positions corresponding to the washing operation. To avoid sample carryover, it uses the so-called 'discretional access inert fluid' (TRAF). The composition of this liquid, which is extremely inert and immiscible with water and which amazingly prevents mixing between liquids successively aspirated by the same pipette, is a patented commercial secret. The aspirating tip used by the analyser is filled with this viscous fluid and also covered with a thin film of it to avoid contact of the material of construction (steel, Teflon) with the liquids handled or the atmosphere. A piston pump effects aspiration and dispensing. Before the sample is aspirated, an air bubble is aspirated to facilitate the introduction of the liquid in the bulk TRAF. The few drops of TRAF accompanying the sample in filling the reaction cup ensure complete sweeping of the sample aspirated in the previous operation. These TRAF drops, which are heavier than water, do not obstruct the passage of light in the subsequent spectrophotometric detection.

Analysers capable of determining several analytes in a single sample require suitable introduction systems. The samples are split into small volumes whose magnitude depends on the particular analyser. Figure 3.7 depicts two of

the most representative types. Batch analysers can use an iterative system
(the probe sequentially fills n analyser cups) or a multi-valve such as that
shown in the figure. The sample is aspirated through a peristaltic pump into
six cylindrically arranged parallel channels connected to one another in the
starting position. As the valve is switched, all six channels are connected to
as many independent carrier or reagent streams which sweep the channel con-
tents to six analyser cups. Continuous analysers, on the other hand, use a
manifold with a sample stream circulating along a main channel with a series
of diversions to various determinative units. This type of configuration,
conceived for the multi-determination of species in clinical chemistry, is
discussed in detail in Chapter 5, devoted to continuous segmented analysers.

a)                                                    b)
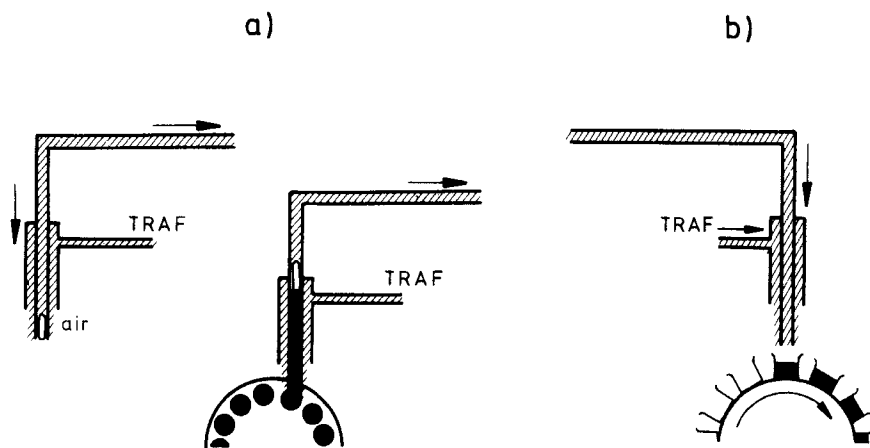


**Fig. 3.6** Transfer of an accurately measured sample volume to the
Technicon RA1000 batch analyser with the aid of a special liquid
(TRAF). (a) Filling position; (b) evacuation position. (Courtesy of
Technicon).

Malmstadt *et al.* [5] reported a liquid sampling system based on measure-
ments of computer-controlled weights (Fig. 3.8). The system uses an electronic
sensor to accurately weigh aliquots of the sample and reagent solutions added
at a given position of the sample tray. Once this operation has finished, the
tray is turned by an appropriate angle to place the receptor cup in the stir-
ring position while another, empty cup is positioned to receive an aliquot.
The volume of liquid added is controlled by the microcomputer —which is later
provided with the exact weight reading for future calculations— through the
corresponding valves.

1)            2)
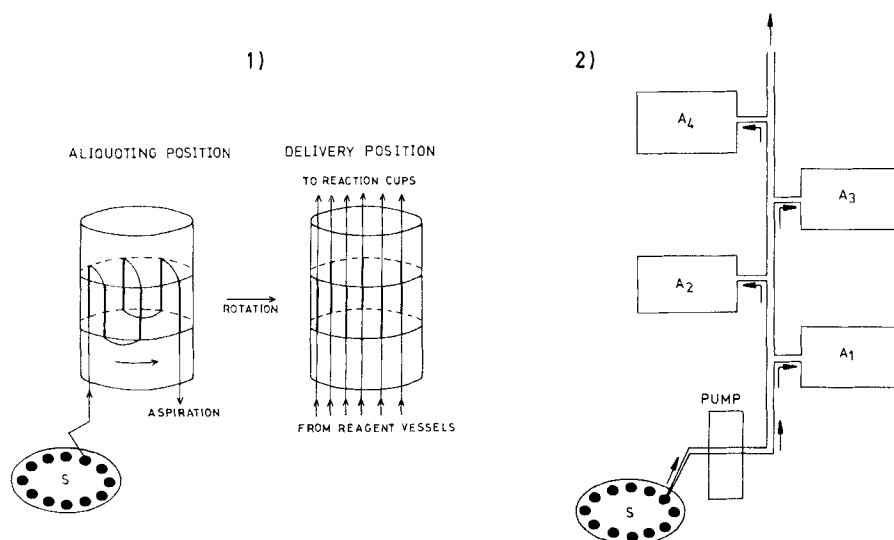


**Fig. 3.7** Sample introduction systems for multi-analyte determinations based on the splitting of the samples into accurately measured volumes. (1) Batch analysers use special multi-injection valves. (2) Continuous analysers employ a manifold directing an accurately measured sample volume to each of the determinative units ($A_1$, $A_2$, etc.).

The sampling of water for the determination of traces of organic pollutants requires a preconcentration step. The use of sorptive pre-columns is a representative example of the inability to separate sample collection and treatment, which are carried out in a single step. This analytical methodology is described in greater detail in Chapter 11, dealing with the automation of chromatographic processes.

Automatic diluters and dispensers are widely used in control laboratories. These are semi-automatic systems working off-line with the analyser or instrument concerned and generally serving the dual purpose of dispensing a given volume of the liquid sample and another of a reagent or diluent in a sequential manner. Hamilton market different models of these devices, the latest of which are furnished with a microprocessor controlling the mechanical motion of two or more high-precision syringes via a step motor. Other models use two peristaltic pumps. Such is the case with the Clinicon diluter, with two independent pumps, one for the sample and the other for the diluent or reagent.

The aspirated and dispensed volumes can be independently programmed (from 10 to 150 µL, from 60 to 900 µL, from 0.2 to 2 mL, etc.). By means of the manually transported aspiration/withdrawal tip, the unknown solution is aspirated by pressing a knob. Then, the volume taken and that of the reagent are poured into the reaction cuvette. Obviously, the sample pump must operate in a reversible fashion.
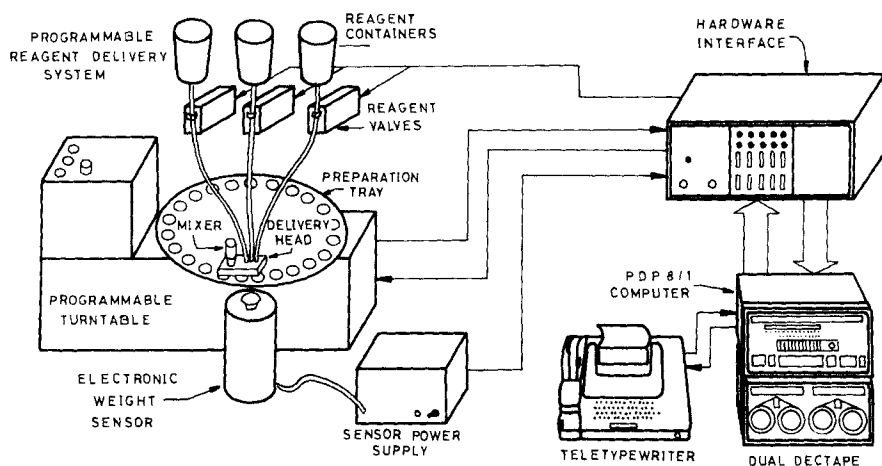


**Fig. 3.8** Automatic system for sampling of variable volumes of liquid by computerized control of weights, reported by Malmstadt *et al.* (Reproduced from [5] with permission on the American Chemical Society).

## 3.5 GAS SAMPLING

The monitoring of environmental pollution requires a large number of determinations to be made on atmospheric samples taken in very different places. The automation of gas sampling systems is highly recommended or even essential in most instances, not only in pollution monitoring but also in industrial product control.

There are two generic types of automatic sampling system, namely *batch* or *intermittent* (off-line) and *continuous* (on-line).

### 3.5.1 Batch gas-sampling systems

These are represented by impregnation, sorption and electrostatic systems,

as well as by those which retain the liquid or solid matter borne by the gas on passage through a filter under the action of an aspirating pump. These systems are subsequently treated as required to introduce the retained analytes into the analyser or instrument used. This operation is carried out manually in many cases, although it can be readily automated in some (e.g. with special sorption tubes which, once the sample has been taken, are placed on a sampler which supplies them to a gas chromatograph incorporating them by programmed thermal desorption). Reed *et al.* [6] developed an automatic sampling system for controlling household air-conditioners. Sulphur hexafluoride is used as tracer and eventually determined by gas chromatography. The rate of change of the gas composition, r, is directly proportional to the logarithm of the tracer concentration (c) at time t and its initial concentration, $c_0$:

$$r = \frac{-\ln(c/c_0)}{t}$$

The sampler mechanism consists of a rotating turntable with 24 vertically positioned, vacuum-sealed 20-mL tubes (Fig. 3.9). The turntable is mounted on a vertical axle which is rotated in steps by a modified uniselector mechanism to place each sampling tube directly beneath a hypodermic needle. This is connected to a small diaphragm pump which is in turn connected to the area of interest. A reciprocating mechanism, driven by a pulsed dc motor, moves the hypodermic needle donwards to penetrate the rubber bung of the sample tube, thereby exposing it to the test atmosphere. After a preset delay, the needle is withdrawn from the self-sealing bung and the turntable is rotated in two steps to position the next sampling tube beneath the needle.

### 3.5.2 Continuous gas-sampling systems

These systems, described in full detail in Chapter 16, uninterruptedly control the concentration level of one or several analytes in evolving gas samples. An interesting example of the implementation of automatic gas sampling is the so-called 'smoking machine', developed by an official laboratory in the UK for monitoring of the toxic substances in tobacco smoke [7]. Cigarettes are placed in a system mimicking the smoker's actions. Figure 3.10 shows squematically the sequential operations performed in the sampling. First, smoke is aspirated with the aid of a syringe. A CF Cambridge filter retains suspended solid particles larger than 0.3 μm in diameter. The smoke taken is exhaled to a flexible collecting bottle with the aid of the syringe and two three-way valves. The sample is introduced into the sensing system. A valve then connects the bottle to conduit fitted to a pump propelling the collected gas to a

continuous infrared detector (NDIR) sensing $CO_2$ and volatile hydrocarbons. The filter is weighed and its contents are extracted with a propanol–ethanol mixture. An aliquot of the extract is introduced into a gas chromatograph for determination of water. The determination of alkaloids is slower as it requires distillation of the above-mentioned extract, a portion of the distillate being analysed photometrically on a Technicon AutoAnalyzer. A central computer controls the automatic operations and collects data from the different instruments (the number of aspirations per cigarette from the smoking machine; the filter weight from a balance; the $CO_2$ and hydrocarbon concentrations from the NDIR detector; the water content from the gas chromatograph; and the alkaloid concentrations from the AutoAnalyzer). Finally, it delivers the complete results corresponding to a cigarette batch. This configuration is a representative example of the combination of manual and automatic operations, although data are handled by a single computer which ultimately supplies the results sought.
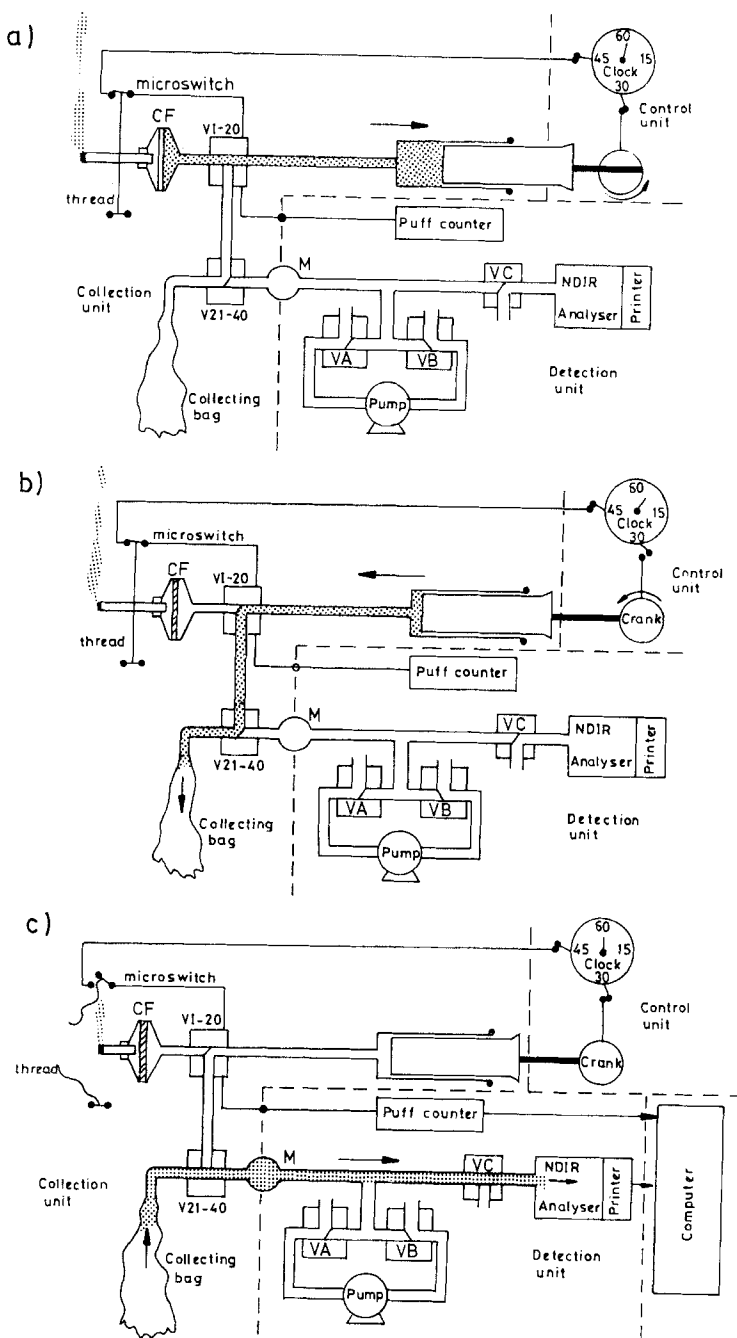


Hypodermic needle

Uniselector
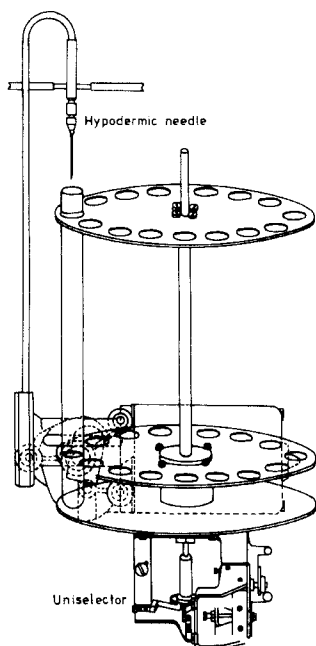
**Fig. 3.9** Automatic gas sampling system. (Reproduced from [6] with permission of Taylor and Francis Ltd).

## 3.6 SOURCES OF ERROR IN AUTOMATIC SAMPLING

The errors introduced by automatic sampling arise from a variety of

**Fig. 3.10** Automatic sampling of tobacco smoke by means of a smoking machine. (a) Puff; (b) exhaust; (c) post-smoking. (Reproduced from [7] with permission of Ellis Horwood Ltd.).

sources associated with factors such as the type of sample handled or the cau-
tion exercised in certain operations (e.g. the cleansing of probes). Although
each sampling system has its own sources of error, there are some generic
causes common to all, the most typical of which (lack of precision, cross-
contamination and mismatching of sample-results) are commented on below.

### 3.6.1 Lack of precision

Errors arising from the lack of precision in  quantitating volumes  (of
liquid or gas samples) or weights (of solids or liquids) are currently minimal
thanks to the advances in micromechanics and microelectronics, which have al-
lowed the development of high-precision systems for the measurement of both
macro and micro volumes. The commonest sources of error when the volume to be
measured is a function of the flow-rate and the time over which collection is
performed (e.g. with peristaltic pumps) are inconstancy of the flow-rate
—flexible tubes must be replaced periodically—, irreproducibility in the
pump's stop-and-go cycles, inaccuracies in the timing devices, etc. The con-
trol of syringes by step motors affords highly precise volume measurements.
For obvious reasons, reproducibility is also indispensable in the movements of
aspirating-dispensing probes: each operation must be carried out over a preset
time interval, during which the probe must be in the appropriate position.
Other errors arising from unpredicted circumstances such as too low levels of
liquid in the vials, which can result in the system taking air rather than the
sample, should be avoided at all costs by using special devices such as opti-
cal sensors.

### 3.6.2 Cross-contamination

*Cross-contamination* or *carry-over*, which is the undesirable mixing of two
successive samples, affects not only automatic, but also manual systems al-
though it is easier to avoid in the latter. Carry-over is a very important as-
pect of autoanalysers, which differ essentially in the manner in which this
undesirable phenomenon is avoided. In assessing the real significance of this
source of error one should take into account that it is a major limiting fac-
tor of sample throughput.

Table 3.2 lists the chief causes of carry-over in automatic analysers. The
phenomenon can originate in two main parts:

(1) In the sample collection system. The magnitude of the effect is  simi-
lar in batch and continuous analysers in this case. It arises from the use of
the same probe and conduits to take samples and/or reagents successively and
can be minimized in four ways:

– By using an intermediate washing solution to flush the  previous sample
from the system.

- By use of TRAF, the 'wonder fluid' employed in the Technicon RA 1000 analysers.

- By aspirating a large volume (one to three times the sample volume used) to sweep the preceding sample out of the conduits when using an injection valve. In practice, this leads to a minimum sample aspiration time.

- By means of external mechanical systems rinsing the liquids (sample or reagent) outside the probe, which must therefore have a position for such a purpose. Some workers use external cleaning systems combining pressurized washing and subsequent mechanical sweeping.

(2) In the transport system, i.e in the transfer of samples and reagents, whether mixed or not, to the detection system. Among batch analysers, only those involving a final transfer to a measuring cuvette or rod stirrers are exposed to the risk. On the other hand, carry-over is a major aspect of continuous analysers. Segmented analysers cannot be freed from this undesirable effect by the mere use of air bubbles; in fact, they require the incorporation of an intermediate washing zone preventing mutual or cross-contamination. The geometric characteristics of FIA assemblies allow the sequential insertion of the samples into the system with no mixing. Robotic analysers are less prone to carry-over, and in this respect they resemble batch analysers without final transfer of the reacting mixture.

Carry-over effects can also be classified according to which ingredients should not be mixed. Thus, in principle, there can be cross-contamination between samples, reagents or both (Table 3.2).

**TABLE 3.2**  Causes of carry-over in automatic analysers

| | |
|---|---|
| According to location | In the sample collecting system |
| | In the analyser |
| | |
| According to the ingredients involved | Between samples |
| | Between reagents |
| | Between samples and reagents |

(a) *Sample carry-over.* This is the commonest source of error in the results provided by analysers dealing with samples in a sequential manner. The greater the difference between the analyte concentration in two samples is, the more marked the effect is. Such an effect can be quantitated by means of

the expression proposed by Dixon [8]. In practice, the extent of carry-over is evaluated by continuously and sequentially introducing into the analyser four standards: two of a high and equal concentration yielding two signals $a_1$ and $a_2$, and another two of low and equal concentration giving rise to signals $b_1$ and $b_2$. The extent of carry-over is determined from the equation

$$k = \frac{b_1 - b_2}{a_2 - b_2} \times 100$$

The operation is repeated several times on different days to obtain an average k value representative of the analyser in question. Most often, k ranges between 0.5 and 2%, which does not significantly influence the results obtained in routine analyses. The test should be repeated whenever anomalously large deviations are observed in the results. For k values greater than 10%, Dixon [8] recommends using the equation above as an algorithm to correct the analytical results after checking the constancy of k with time. For a given k value, the influence of carry-over depends on the difference in concentration between the analytes in the successive samples. Thus, if k $\equiv$ 1% and the analyte provides two signals $S_1$ and $S_2$ of relative magnitude 100 and 10, the error made will be 0.001% in $S_1$ and 10% in $S_2$ (i.e the lower the analyte concentration, the more marked is the effect).

(b) *Reagent carry-over* is typical of batch analysers capable of determining several analytes in a single sample (e.g. clinical determinations). In this case, carry-over arises from the use of the same probe to dispense the different reagents ($R_1$, $R_2$, $R_3$...) to the corresponding reaction cups. The effect of this type of carry-over is not a result of the volume change (dilution), which is virtually negligible, but of the chemical influence of one of the reactants on the assay involving another and taking place in the next cup. Such chemical influence can be exerted through changes in the pH or, worse, as an inhibitory or activating effect on a catalysed enzymatic reaction which may result in large errors disproportionate to the extent of carryover, which must therefore be determined by a special procedure such as that reported by Broughton [9] and involving the duplicate determination of a series of analytes in the same sample by different assays carried out continuously and sequentially, e.g.

      - Test 1 (reagent $R_1$) ... concentration of analytes $a_1$ and $a_2$

      - Test 2 (reagent $R_2$) ... concentration of analytes $b_1$ and $b_2$

      - Test 3 (reagent $R_3$) ... concentration of analytes $c_1$ and $c_2$

The error made in the determination of $b_1$ arising from the contamination resulting by reagent $R_1$ in test 2 can be expressed in relative form as

$$\frac{b_1 - b_2}{b_2} \times 100$$

if the second determination is assumed not to be subject to contamination by the preceding reagent. As the differences encountered in duplicate determinations can be due to random errors, carry-over between reagents should be considered to be significant only if replicate differences for a given sequence are all in the same direction (i.e all positive or all negative) and if all differences are greater than twice the within-run coefficient of variation for that analyte concentration.

In order to avoid carry-over as far as possible in multi-determinations with batch analysers, the determinative sequence chosen should be that which results in the smallest effects possible from the preceding reagents.

(c) *Reagent-to-sample carry-over* may be the result of the probe coming into contact with the reacting mixture before being used to aspirate the next sample. The influence of the reagent contamination is significant if it is exerted on a different assay. However, if it is the same assay which is applied to different samples, such an influence is negligible, although there might be some extent of carry-over between samples. If, on the other hand, different assays are carried out on the same sample, the effect can be cumulative. The extent of reagent-to-sample carry-over can be evaluated by the procedure developed by Smith *et al.* [10], who used a dye as reagent and water as the sample. If any dye is detected in the following sample, carry-over should be minimized or taken account of.

### 3.6.3 Mismatching of sample results

Of the three major sources of error found in automatic sampling, this is no doubt the most important from a practical point of view. Dealing with a large number of samples involves the risk of their erroneous matching with the results. One such erroneous identification is sufficient to completely mismatch the whole series with the actual results. This risk is particularly serious in clinical chemistry —for example, a patient with a diabetic coma might be assigned the blood glucose data from a patient with normal levels and the latter patient in turn being diagnosed as having a serious disease.

For obvious safety reasons, sample identification systems are mandatory in this field. Keller [11] defined the term 'sample identification' as "all instructions, equipment and processes for the recognition and allocation of a specimen or sample, and an analytical result to a distinct source of specimen." The identification involves three essential aspects: (a) allocation of a complete identification statement consisting of information about the source

of the sample, kind of material, ordered tests and instructions, and also the timing; (b) intra-laboratory identification, which involves generating and identifying symbols fixed to the sample during all transport and transfer processes; (c) a system for permanent control of identity throughout the analytical process —including the final stages of the calculation and delivery of results.

The identification mechanism should be started as soon as the raw sample enters the analytical process. Immediately on admission to large hospitals, patients are given an identification letter including basic initial data for identification of his/her blood or urine samples submitted to the laboratory for analysis, together with the name of the doctor who ordered the analyses, the analytes to be determined and the place where the patient is located. The use of computerized systems for this purpose is obviously of great assistance. There are two main procedures available for sample identification in laboratories. Indirect procedures, now on the decline, involve positional identification of the sample according to its place in a chain or on a turntable —a previously made list is used as a key to match the results with the samples. This type of procedure carries the greatest risk of sample interchange in the laboratory.

Direct identification methods are more recommended and can be implemented in two ways. One involves marking the sample container and introducing it directly into the analyser, equipped with an electronic device for identification which, with the aid of the microprocessor, allows the continuous monitoring of the sample and guarantees absolute matching between samples and results. This is the cheaper and safer way. When the complete identity statement is too extensive and contains more information than is required for the analytical process, it is necessary to abridge the statement to an identification symbol with the aid of a code. Sample containers, process vessels and analytical records are linked to one another by the identification symbols. Retrieval of the complete identification statment calls for a decoding process in which computers are a key component, particularly in large hospitals or industries.

There are a variety of systems for direct identification (labelling) of the tubes and other vessels used to hold samples. The Eppendorf labelling system is based on reflection marks imprinted on the process vessels, which are read automatically by the machine; they are supplemented by visual characters so that visual checking by the operator is possible at any time. The marks can be printed on labels or punched into perforated cards. Bar codes are a recent alternative now in widespread use. They offer a series of advantages such as the simplicity and rapidity of the reading or identification (based on laser technology). Even more recent and promising are optical character rec-

ognition (OCR) systems, capable of directly reading each of the digits on the label.

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# 4
# Automation in sample treatment

## 4.1 INTRODUCTION

The concept of 'sample treatment' is normally used to refer to the series of operations aimed to render the collected sample ready for introduction into the analyser or instrument. Although this should be a distinct stage, very often it is not separable from the analytical reaction development, either because both are carried out simultaneously (e.g. liquid–liquid extraction with formation of a coloured chelate) or because they take place in a sequential manner within the analyser.

As put forward in the preceding chapter, it is difficult to establish a clear-cut distinction between the automation of sample collection and treatment in many configurations, both commercial and non-commercial. Thus, in collecting air pollutants by means of sorption tubes, the analytes are simultaneously preconcentrated from the collected samples.

The objectives of the automation of the sample treatment stage are as varied as the nature of the sample and the number and type of analytes it may contain. The commonest, summarized in Table 4.1, are as follows:

(a) Giving the sample the treatment best suited to its nature by applying to it the most appropriate preparative technique, *viz.* dissolution, extraction, filtration and so forth.

(b) Preconcentrating trace analytes in those cases where the determinative analytical technique to be applied features a detection or quantitation limit much higher than the actual analyte content(s) in the sample. Preconcentration is thus an indirect way to increase sensitivity.

(c) Removal of disturbances due to other matrix components which might interfere with the determination of the analytes. This, in turn, increases the selectivity indirectly.

(d) Facilitating the analytical determination, otherwise unfeasible without a major alteration in the sample characteristics (e.g. a change from a polar to a non-polar solvent or vice versa).

(e) Protecting the analytical system from the potential deterioration caused by the matrix components.

(f) Facilitating the development of the analytical reactions for detection of the analytes.

(g) Transferring the treated sample to the detection system.

**TABLE 4.1**

Chief objectives of automation in sample treatment

---

. Preparation of sample
  (dissolution, disaggregation, extraction, filtration)
. Preconcentration
. Interference removal
. Facilitating the determination
. Facilitating the development of the analytical reaction
. Transfer to the detection system

---

Analytical separation techniques play a major role in the above-mentioned preliminary operations. Their implementation on automatic systems can be achieved in a variety of ways, although most often it is done in one of two ways, namely discontinuously or *off-line* and continuously or *on-line*. The former is better suited to continuous analysers (SFA, FIA), and the latter is equally suited to continuous and batch analysers.

The following sections deal with the automation of sample treatment from the point of view of the particular separation technique used. Each section presents representative examples rather than a comprehensive coverage of the topic, which on its own would be subject of a separate book.

Figure 4.1 illustrates some of the ways in which the chief preliminary operations (sample collection and treatment) can be connected to each other and their relationship to the instrument or analyser. Complete automation is achieved when no clear distinction can be established between all three stages. Human intervention, on the other hand, is minimal in on-line automation (e.g. via a sampler). Finally, off-line configurations involve the independent development of each preliminary operation in an automatic module liable to be connected on-line to a sampler; however, in some of the connections, samples are transferred manually. Table 4.2 lists some of the different possibilities resulting from the various connections and separation techniques, illustrated with examples corresponding to the different sections of this chapter.
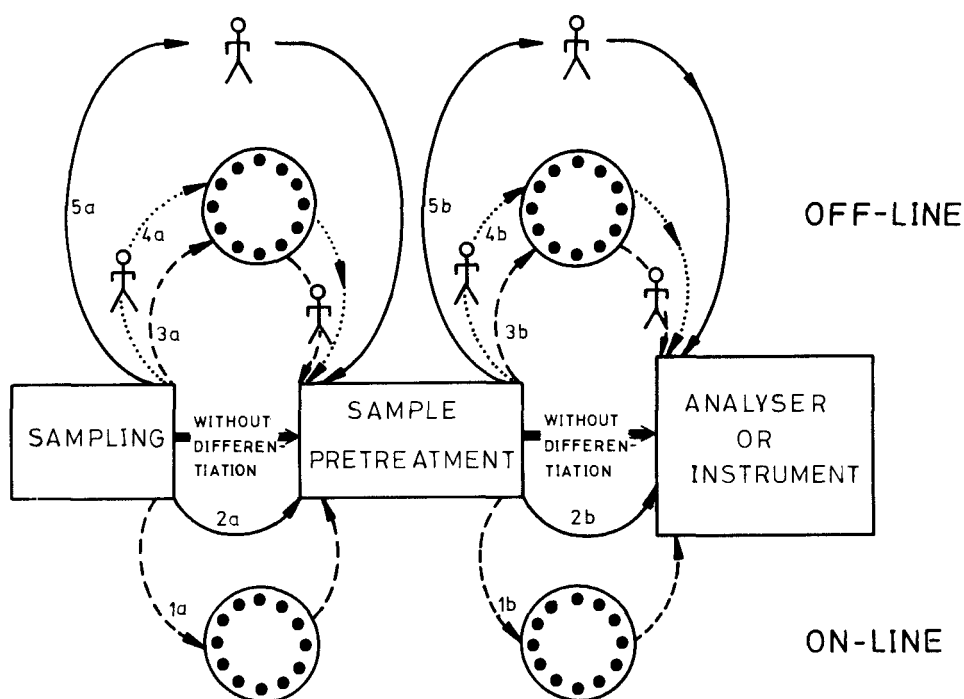
**Fig. 4.1** Automation relationship (off–line, on–line) between the sam-
ple collection and treatment and its introduction into the analyser.

## 4.2 DISSOLUTION AND DIGESTION

The automation of preliminary operations involving a solid sample or a
liquid sample containing a suspended solid is far from easy, so much so that
the elimination of human intervention in this tedious stage is, understand-
ably, of great interest. The best alternative to the automation of solid sam-
ple treatment is the use of robots mimicking the operations carried out manual-
ly by an operator (see Chapter 9).

Systems requiring only partial dissolution of the sample call for lixivia-
tion rather than dissolution or digestion. Such systems are dealt with in Sec-
tion 4.9, devoted to solid–liquid extraction.

The term 'automatic' is improperly applied to a host of commercially avail-
able instruments for the dissolution and digestion of several samples (from 6
to 24) simultaneously. These are straightforward off–line configurations con-
sisting of a central heating unit equipped with time and temperature controls
and —optionally— a stirrer, where suitable vessels to which quantized sam-
ples have been previously added manually are placed, also manually. Some de-
signs also feature automatic addition of reagents prior to, during or at the

end of the process. The incorporation of the resultant solution into the in-
strument or analyser is generally carried out manually, although it is also
liable to automation.

**TABLE 4.2**

Different configurations described in this chapter illustrating the relation-
ships established in Fig. 4.1

| Configuration | Pretreatment | Figure No. |
|---|---|---|
| 4a–3b | Dissolution | 2 |
| 4a(5a)–2b | Distillation | 7 |
| 5a–5b | Distillation | 5 |
| 4a(5a)–2b | Filtration | 11 |
| 3a–3b | Sorption | 12 |
| 2a(4a)–2b | Sorption | 13 |
| 2a(4a)–2b | Ion exchange | 15 |
| 4a(5a)–2b | Gas diffusion | 17 |
| 1a–1b | Solid–liquid extraction | 19 |
| 4a–3b(1b) | Liquid–liquid extraction | 22 |
| 1a–2b | Liquid–liquid extraction | 27a |

   The pretreatment of solid samples for the determination of metal traces
and ultratraces by ICP atomic emission spectroscopy is a delicate stage call-
ing for automation. Jobin-Ybon have developed an apparatus called a Plasmasol
in which samples are introduced manually and individually treated, and where-
from the resultant solution is drawn manually to be diluted —also manually—
prior to introduction into the instrument. In this semi-automatic predissolu-
tion design, the sample and flux mixture are poured manually into a composite
crucible with two recipients, one external of platinum and another removable
inner crucible made of vitreous carbon. The chief advantage of the special
crucible is the absence of sample wetting. The crucible is located in a fur-
nace which can be moved about a horizontal axis. Heating is accomplished by
high-frequency induction. The furnace is fixed on a rotary disc which rotates

about an axis passing through its centre. The pretreatment can be programmed to be carried out at a given temperature during a preset time, or even as two independent stages. At the end of the fusion, the furnace continues to turn until it comes into the pouring position. The crucible then rotates 120° and the highly mobile molten product is poured into a beaker on a magnetic stirrer which contains about 100 mL of dilute acid. The stirrer is activated at the moment of pouring and the fusion product is finely divided in the solution. After a set time, the furnace returns to its vertical position, the magnetic stirrer ceases to act and a new sample preparation cycle is started. The beaker is placed manually on a hot-plate for 3–5 min in order to terminate the dissolution process. All these functions, including fusion, furnace agitation, pouring, return of furnace to the initial position and magnetic stirring of the final solution, are automatic.
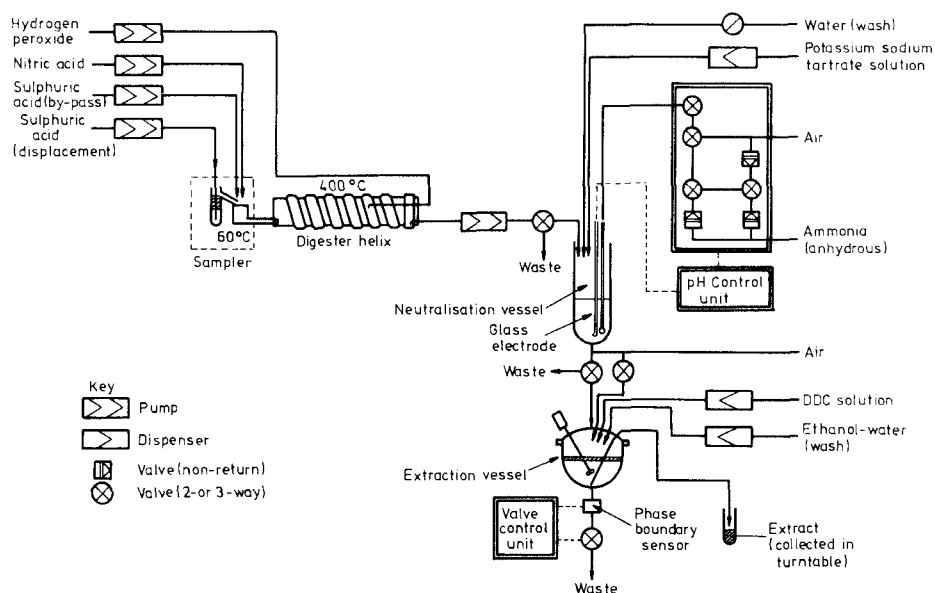


**Fig. 4.2** Scheme of automatic system for the determination of metal traces in foodstuff including sample dissolution. (Reproduced from [1] with permission of the Royal Society of Chemistry).

Technicon's SOLIDprep II module, described in Chapter 3, is a representative example of automatic sample dissolution systems where up to 20 samples held on a turntable can be treated sequentially. An aliquot of the resultant

solution is taken automatically by an aspiration probe and introduced into a continuous analyser. This module thus features automatic operations preceding and following dissolution proper.

Similar to the SOLIDprep is the automatic system developed by Jackson *et al.* [1] for the determination of metal traces in foodstuff. As can be seen from Fig. 4.2, it consists of the following operational units:

(a) A tray for preweighed solid samples held in specially designed vials. The entire unit is thermostated at 60°C. In the sampling position, the side tube of each vessel is connected to the digestor via a small open reservoir.

(b) A system dispensing the digestion reagents. Sulphuric acid is added at two points, namely to the vial to flush the sample and to the reservoir, which also receives some nitric acid. A hydrogen peroxide stream is inserted into the centre of the digestion unit.

(c) A continuous digestion unit consisting of a borosilicate glass helix which is rotated over three banks of heaters. The sample and digestion acids are fed in at one end and transported over the heaters by rotation of the helix, and the resultant digest is pumped out of the other end through a pneumatically actuated valve, either to the neutralization vessel or to waste, by means of an oscillating piston pump.

(d) A neutralization unit consisting of a vessel and a system for dispensing anhydrous ammonia gas under the continuous control of a pH meter regulating the addition. The vessel is automatically flushed with the aid of a water stream.

(e) An automatic liquid-liquid extraction system with paddle stirring. This receives the contents of the neutralization unit through a control valve which can also be switched to divert the flow to waste in the flushing operation. The extraction vessel also receives programmable volumes of a solution of diethyldithiocarbamate in 2-heptanone. After stirring for a preset time and phase decantation, the organic phase is drained by a suction system leading it to one of the vials of a fraction collector. Draining of the heavier phase is also under control. An ethanol-water stream is used to flush the system. A more detailed description of a similar extraction system is given below (see Fig. 4.23).

In short, the system developed by Jackson *et al.* [1] features automatic on-line sample collection from a turntable, acidic-oxidizing digestion of the sample, neutralization, liquid-liquid extraction and introduction of the extract into a sampler vial.

## 4.3 VOLATILIZATION

This separation technique is based on the formation of a gas phase. Al-

though some workers use this term indiscriminately, a distinction should —to our minds— be made between volatilization and distillation depending on whether the starting phase (sample) is solid or liquid.

Further distinctions can be made between the two separation processes according, for example, to whether the separation is effected with heat or not, whether a reagent generating the gas phase is added or not, and whether the species of interest passes into the gas phase or not. All these alternatives involve major differences in the corresponding automatic systems required. This section deals exclusively with the automation of methodologies based on the formation of a gas from a solid. As the analytes involved in these cases are generally volatile, the sample treatment system must be prepared to collect the vapours emitted upon addition of a reagent or heating of the sample. Head space gas chromatography is a representative example: a solid held in a closed vial is heated in a controlled fashion while the chromatographic carrier gas is forced to circulate through it. In this way, the volatile analyte mixture is incorporated into the chromatographic system. A detailed description of this accessory for on-line use with gas chromatographs is given in Chapter 12.
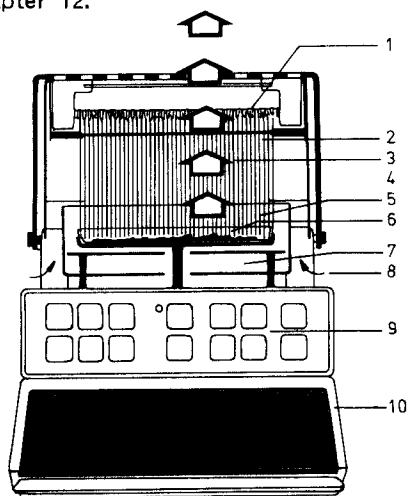


**Fig. 4.3** Automatic system for the determination of humidity in solid samples (Mettler IR LP 16 desiccator. (1) Pair of dual infrared heating bars; (2) two thermal probes; (3) rising humidity; (4) infrared radiation; (5) air-cut; (6) sample scale; (7) air gates; (8) outside air; (9) input keyboard; (10) high-precision balance. (Courtesy of Mettler).
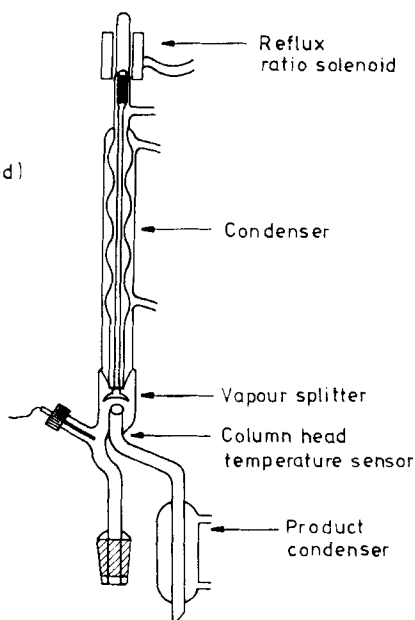
The routine determination of the water content in a variety of samples is of great interest in areas such as agriculture and nutrition. Several manufac-

a)
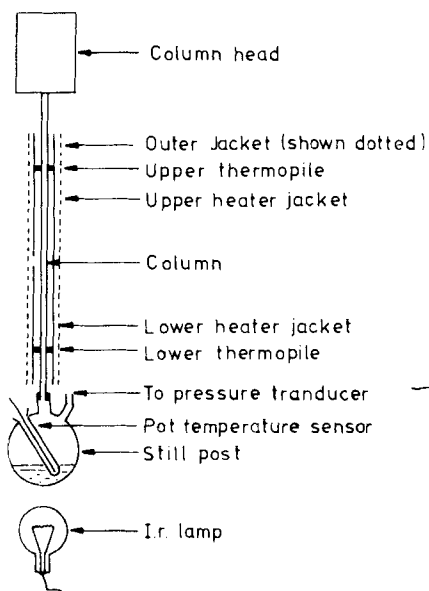
b)



**Fig. 4.4** Automation of a laboratory fractional distillation process. (a) Distillation column; (b) column head. (Reproduced from [2] with permission of Elsevier).

turers of analytical balances have developed automatic systems for this purpose by simply fitting accesories to existing models. Such is the case with the LP 15B module, designed as an accesory for the Mettler PE 300 balance. In the resulting microprocessor-controlled assembly, depicted in Fig. 4.3, the sample is placed in the balance scale, located in the accesory furnace. Four infrared raditation bars are automatically activated as the furnace lid is re-placed. A 12-stop switch allows the selection of the heating intensity, characterized by its remarkable evenness. The temperature can be selected in 5° intervals from 50 to 160°C via the front panel. The heating time is equally programmable between 1 and 240 min. The data generated are automatically processed to deliver results in the required form: either as a percentage humidity, referred to the wet sample, or in ATRO values if the final weight is taken as a reference. This assembly for the determination of humidity can also be regarded as a specific automatic batch analyser, described in greater detail at the end of the next chapter.

## 4.4 DISTILLATION

Separation proceses based on liquid-gas interfaces can be divided into two major groups according to the manner in which they are connected to the analyser or instrument in their automation, namely *off-line* and *on-line*. Each of these groups can be in turn divided into sub-groups according to the analyte volatility and the use of heating and/or a reagent.
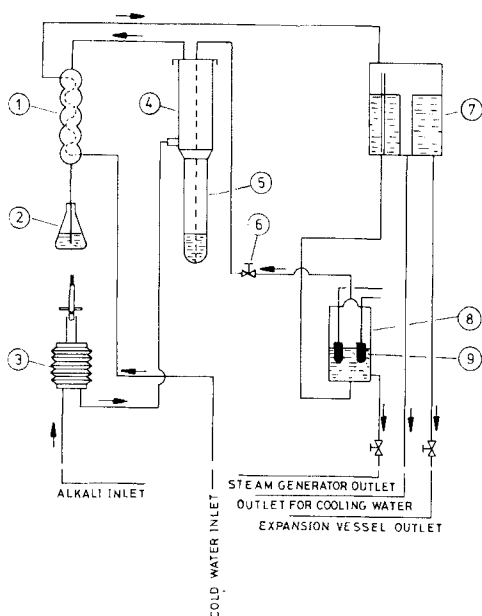


**Fig. 4.5** Scheme of the Tecator KjelTec semi-automatic distillation unit 1 for steam distillation of ammonia. (1) Condenser; (2) receiver flask; (3) alkali pump; (4) splash head; (5) digestion tube; (6) steam valve; (7) expansion vessel; (8) steam generator; (9) electrodes. (Courtesy of Tecator).

### 4.4.1 Off-line distilation systems

In these, the gas phase is suitably collected and subjected to the subsequent analytical determinations in a discontinuous fashion. Although the classical distillation systems have fallen into disuse since the advent of the advantageous gas chromatography, their automation has fostered the development of assemblies of some interest. Chipperfield *et al.* [2] reported a computer-controlled laboratory fractional column for small-scale preparations in which a microcomputer controls the column-jacket temperatures, boil-up rate and reflux ratio to achieve optimum separations. A schematic diagram of the dis-

tillation column and column head is shown in Fig. 4.4. The assembly uses a number of continuous control systems, namely: a pot temperature sensor, a pressure transducer, two 15-junction copper-constant thermocouples located at the ends of the column, a column head temperature sensor and a reflux ratio solenoid, whereby the traditional problems associated with manually controlled fractional distilation (e.g. temperature control in different parts of the system, continuous attention by the operator) are avoided. The microcomputer is used to adjust the boiling rate, various temperatures at different points and the reflux/take-off ratio for separations over a wide range of temperatures. In addition to acting as a controller, the computer monitors the distillation process and displays a schematic diagram of the distillation apparatus and the temperatures at the still pot and the condenser.

The determination of nitrogen in a variety of materials based on ammonia distillation (Kjeldahl methodology) is of great analytical interest in some fields. Figure 4.5 shows the scheme of the KjelTec I instrument marketed by Tecator. Once homogenized —and ground if necessary—, the samples are weighed and introduced into digestion tubes to which the reagents transforming nitrogen into ammonium ion (an acid, an oxidant and a catalyst) are added. These tubes are heated at 420°C in a preheated digestion unit for a suitable time and the emitted gas is collected in individual vessels in which, once cooled, it is diluted and introduced into the distillation unit. By manually exerted pressure, a preset volume of concentrated sodium hydroxide is then added. Next, the steam valve is opened for a preselected time (about 5 min) and the steam is passed through the sample in order to sweep the ammonia gas through a condenser to a receiver flask containing boric acid. The analyte is titrated off-line manually in each collecting flask. A higher degree of automation is represented by the KjelTec Auto 1030 model, in which, once the sample has been introduced, all subsequent operations —including the terminal acid-base titration, the end-point of which is detected photometrically with the aid of an acid-base indicator contained in the boric acid solution— are performed without human intervention. Obviously, the system includes a system for automatic draining and cleaning of the receiver flask, which is thus rendered ready for collection of the next sample. The scheme of this analyser, which can be regarded as an on-line analyser, is depicted in Fig. 4.6.

### 4.4.2 On-line distillation systems

In these configurations, the vapour or residue containing the analyte(s) is introduced continuously into the instrument or analyser. A distinction should be made depending on whether the analyte of interest is volatile or not.

When it is the vapour phase which is to be collected, the system uses a re-
agent to generate or release the volatile species, which are led directly to
the analyser. Below are described two examples differing in the need for heat-
ing, compulsory in one and dispensable in the other.
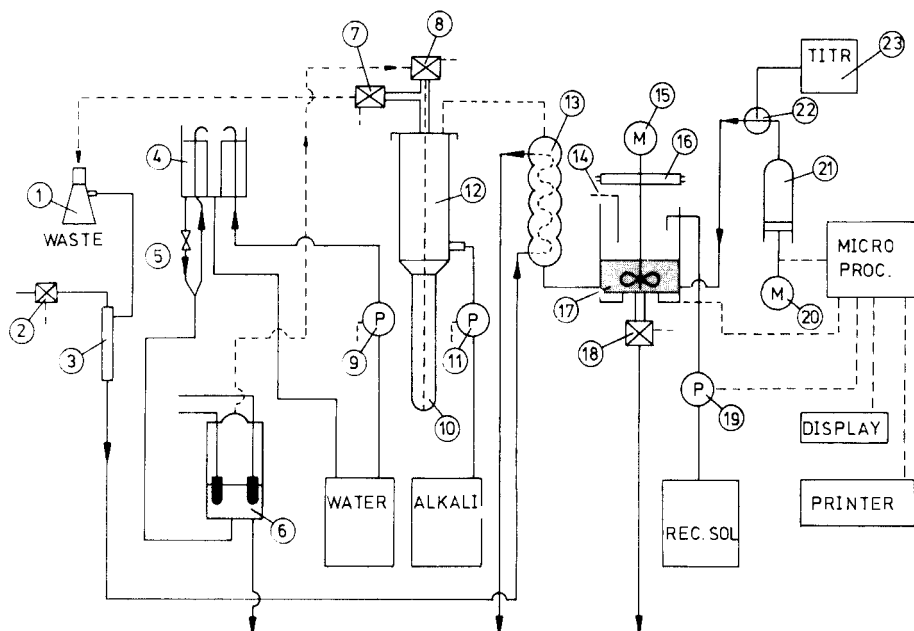


**Fig. 4.6** Tecator KjelTec Auto 1030 automatic analyser including
distillation and photometric acid-base titration of ammonia. (1) Waste
flask; (2) water (inlet) valve; (3) water aspirator; (4) expansion
vessel; (5) feed valve; (6) steam generator; (7) vacuum valve (if tube
drainage); (8) steam valve; (9) water pump; (10) digestion tube; (11)
alkali pump; (12) distillation head; (13) condenser; (14) level pin;
(15) motor (stirrer); (16) lamp (titration vessel); (17) titration
vessel; (18) drain valve (titration vessel); (19) receiver solution
pump; (20) motor (burette); (21) burette cylinder; (22) three-port
valve; (23) titrant storage flask. (Courtesy of Tecator).

Pihlar and Kosta [3] have developed a continuous distillation system for
the amperometric determination of cyanide in water with the aim of avoiding in-
terference from other electroactive species. The configuration is depicted in
Fig. 4.7. A peristaltic pump sets the flow of sample, which is continuously as-
pirated, and that of the different reagents, in addition to regulating the two
outgoing streams of the system. The sample, acidulated with a phosphoric acid
stream, is introduced into the distillation unit, also shown in Fig. 4.7. Its

lower part includes an electrical heating system while the central one has a water cooling system. The liquid phase is drained through its bottom into a cooler leading to waste through a peristaltic pump which regulates its flow. The released hydrogen cyanide is carried by a nitrogen stream into the absorption column after merging with a stream of sodium hydroxide solution. The basic analyte solution emerges from the bottom and is driven to the continuous electrochemical detector through the peristaltic pump. A debbubler allows the elimination of the nitrogen introduced into the system. Cyanide is thus quantitatively recovered except in the highest concentration range (100–1000 mg/mL) and can be determined at concentrations between $10^{-3}$ and 1 mg/L and a sampling frequency of 60 $h^{-1}$.
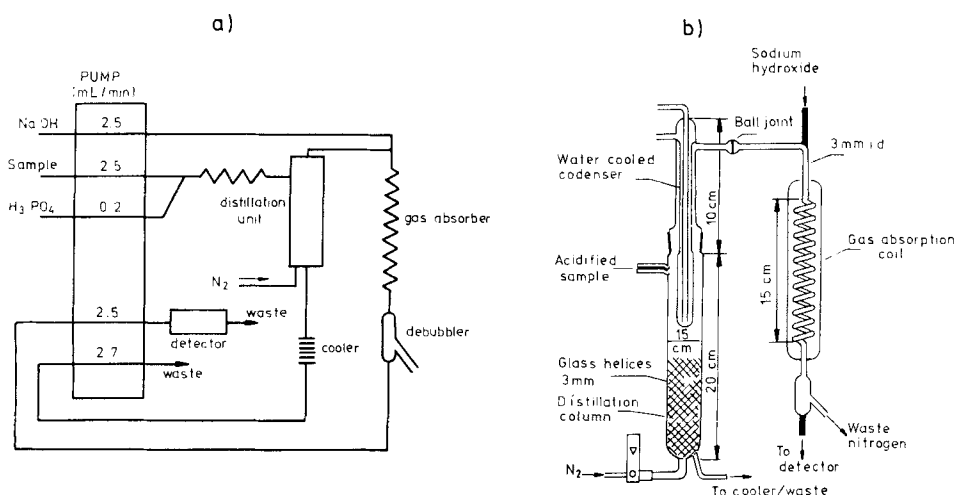


**Fig. 4.7** On-line distillation system for the amperometric determination of cyanide in water. (a) Diagram of the continuous system for establishment of the different streams. (b) Details of the distillation and absorption units. (Reproduced from [3] with permission of Elsevier).

There are several commercial accessories available with different degrees of automation for hydride generation as an on-line preliminary stage in the determination of species by non-flame atomic absorption spectroscopy. An interesting alternative on account of its simplicity involves generating the hy-

dride in a continuous fashion in an FIA system suitable for this purpose. Figure 4.8 depicts the system developed by Astrom [4] for the determination of bismuth traces (10-50 µg/L). The sample is injected into a hydrochloric acid solution that is mixed with a basic sodium borohydride stream. A stream of nitrogen is introduced after the reaction coil in order to facilitate the release of the volatile metal hydride. The gas-liquid separation takes place in a continuous manner in a separator resembling the debubblers used with continuous air-segmented analysers. The gas is driven to a quartz cell located in the light path of the atomic absorption spectrometer used. The flow-rate of the stream emerging from it is regulated by the peristaltic pump, thereby ensuring even functioning of the gas-liquid separation. The automation of this preliminary operation considerably increases the sampling frequency (up to 100 $h^{-1}$), sensitivity and selectivity in comparison with the manual procedure for hydride generation. This system requires no heating.
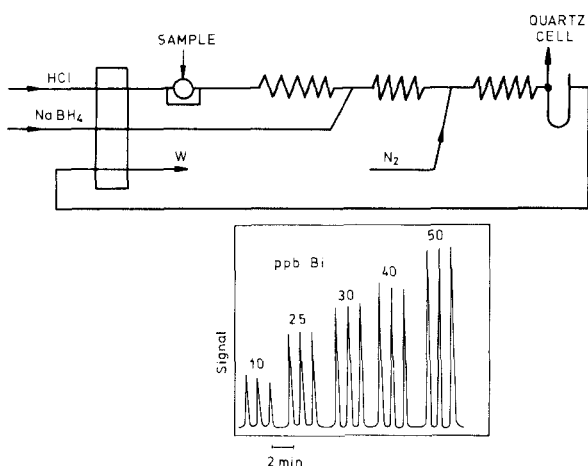


**Fig. 4.8** FIA assembly for continuous generation of hydrides and transport to the quartz cell of the atomic absorption spectrometer, designed for the determination of bismuth traces. (Reproduced from [4] with permission of the American Chemical Society).

When a non-volatile analyte is dealt with, the vaporization of a liquid phase can be used to remove interferences or facilitate the analytical technique by changing the solvent. Such is the case with the Evaporation-to-Dryness Module (EDM) developed by Technicon, the operational diagram of which is depicted in Fig. 4.9. The liquid sample is aspirated through a peristaltic pump and poured slowly on a Teflon conveyor belt. For a preset time, a zone of the module is subjected to extreme vacuum to effect the evaporation of the

original solvent. The dry sample, placed on the Teflon belt, is dissolved on passing by a zone where a stream of another solvent flows upwards and the solution is continuously aspirated to a sampler or, directly, to the instrument or analyser. This module is very useful for solvent change-over in liquid chromatography (e.g. to suit the sample to reversed-phase chromatography) and for introduction of samples into instruments with serious constraints on certain solvents (e.g. aqueous samples in mass spectrometers).
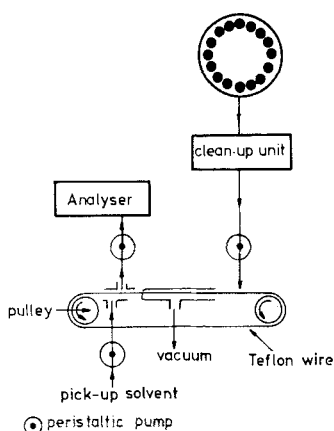


**Fig. 4.9** Continuous solvent change-over system for vacuum distillation and redissolution marketed by Technicon under the trade name Evaporation-to-dryness Module. (Courtesy of Technicon).

## 4.5 FILTRATION

The filtration operation can have two uses in automatic analytical processes depending on the source of the solid material to be filtered out:

(a) When this is initially present in a liquid or gas sample, more or less finely divided, filtration can be aimed to collect and preconcentrate the material in order to use it as such to carry out the analyte determination or simply to clean the material. The automatic systems for the collection of airborne particulates and the filtering units used in continuous water collection systems are representative examples.

(b) When the solid matter is generated within the analytical process as a precipitate, the aim is usually to facilitate the analyte determination or its preconcentration.

Filtering systems can be roughly divided into continuous and discrete. The former, much more interesting with a view to automation, are the subject of the remainder of this section; automatic discrete filtering is carried out chiefly by robot stations and a few automatic systems (see Fig. 4.21).

It should be noted that few continuous filtering systems have been incor-
porated into automatic systems so far, probably because of the difficulties in-
volved. All such systems involve the generation of a precipitate containing
the analyte, which can be determined indirectly via the reagent (differential
method) or directly in the precipitate itself.

Figure 4.10 depicts the continuous precipitation system incorporated into
a continuous segmented analyser, developed by Skinner and Docherty [5] in
1967. It was conceived for determination of potassium in fertilizers and is
based on the continuous monitoring at 266 nm of the absorbance of the excess
of precipitating reagent, *viz*. sodium tetraphenylborate (STPB), after complete
precipitation of the analyte. The stream resulting from the merging of the re-
action ingredients (the sample, sodium hydroxide, formaldehyde to destroy am-
monium ions and STPB) is passed through an ultrasonic bath improving the prec-
ipitate characteristics and preventing its build-up in the system. A piece of
filter paper moving virtually perpendicularly to the flow continuously removes
the precipitate formed. A magnetically stirred mixing minichamber (2 mL inner
volume) is essential to avoid anomalies in acquiring the photometric results
(absorbance decrease). The results obtained with the system for the determina-
tion of the potassium oxide content of fertilizers compare well with those
found by the traditional flame photometric technique.
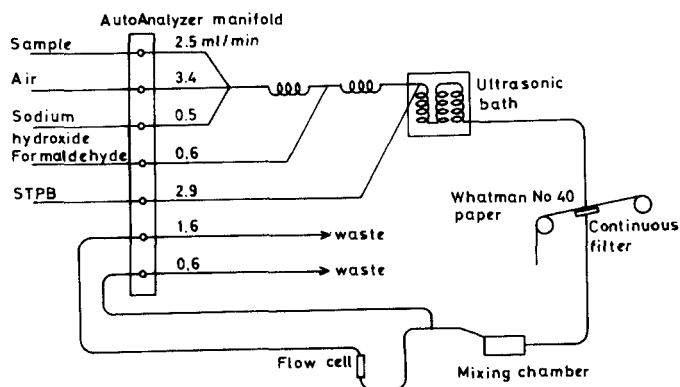


**Fig. 4.10** Scheme of continuous segmented analyser with continuous
filtering system for the determination of potassium in fertilizers.
(Reproduced from [5] with permission of Pergamon Press).

Continuous precipitation can be implemented more simply in an FIA system
designed for the indirect automatic determination of anions by atomic absorp-

tion spectroscopy. In Fig. 4.11 are depicted two systems designed for this pur-
pose by the authors' team [6]. They are based on the retention of the precipi-
tate formed in the reaction coil by one the filters typically used in HPLC for
continuous solvent cleaning, the scheme of which also appears in the figure.
The system can operate in two modes:
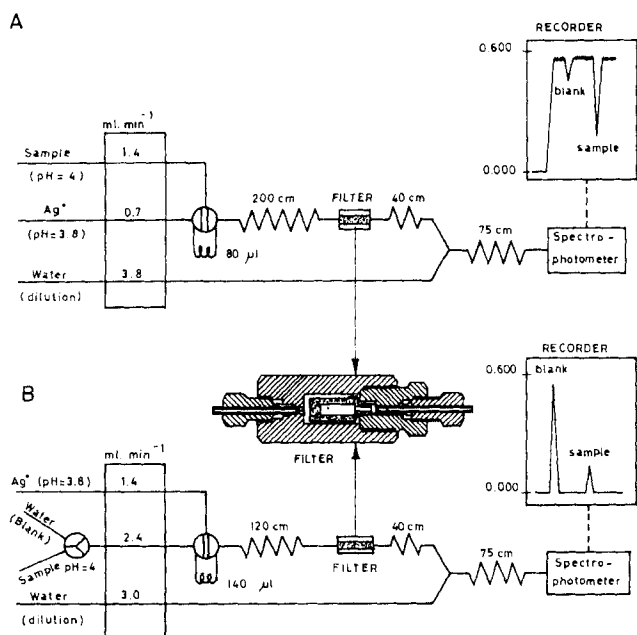


**Fig. 4.11** Continuous filtering systems for use in FIA for the indi-
rect determination of anions by atomic absorption spectroscopy. (A)
Without precipitate dissolution; (B) with precipitate dissolution.
(Reproduced from [6] with permission of the Americal Chemical Soci-
ety).

(a) Without precipitate dissolution. The anion-analyte is injected into a
stream of the reagent-cation. On starting the peristaltic pump, the cation is
driven directly to the nebulizer and a constant signal is obtained as a re-
sult. The injection of a distilled water blank gives rise to a small negative
peak. Subsequent injection of the solution containing the anion results in the
gradual disappearance of the precipitating reagent from the zone where precipi-
tation takes place, which in turn causes a momentary decrease in the signal,
the height of which is directly proportional to the reagent concentration. The
filter requires cleaning in an ultrasonic bath after 50-400 injections, depend-
ing on the nature of the precipitate and the analyte concentration.

(b) With continuous dissolution of the precipitate. The precipitate is formed and retained as in the previous case. Two synchronized diverting valves denoted M and S in Fig. 4.11, allow a stream of washing solution and another of an acid or a solvent for the precipitate dissolution to be passed through the filter. The continuous recording of the signal shows a falling portion corresponding to the washing and a typical FIA peak corresponding to the precipitate dissolution. The height of the latter is proportional to the analyte concentration.

Both continuous precipitation systems were assayed on three types of precipitate, namely gelatinous ($Fe_2O_3 \cdot H_2O$) for the determination of ammonia, curdy (AgCl) for the determination of chloride and crystalline ($CaC_2O_4 \cdot 2H_2O$) for the determination of oxalate. Recoveries close to 100% were obtained in all cases, even at low analyte concentrations, with few interferences. The assembly in Fig. 4.11b, although more complex, has the advantage of requiring no blank.

## 4.6 SORPTION

The use of finely divided solid materials for the retention or sorption of analytes or interferents in a liquid sample passing through them is a common alternative to sample pretreatment on account of the advantages offered, namely:

(a) It affords trace preconcentration in a most convenient and efficient manner. There is no limitation, in principle, to the sample volume to be treated, which is a substantial advantage over other separation techniques such as extraction or voltammetric stripping.

(b) It is of great use in removing interferents (sample clean-up).

(c) It is suitable for sample storage. Thanks to the inert nature of most sorbents, the retained species remain unchanged for long periods, which is of outmost importance in off-line determinations.

(d) It exerts a protective action (e.g. in filtration) in preventing undesirable species, whether solid or liquid, from reaching the analyser or instrument and causing malfunctions —with potential economic repercussions.

(e) Its automation poses no serious technical difficulties.

The sorbents most frequently employed are silica, alkylsilane-modified silica (bonded phases), alumina, porous polymers (with and without ion-exchange groups) and carbon materials. They are usually employed packed in cartridges or short stainless steel or glass columns. Depending on the physical properties of the sorbent (*viz.* particle size, bed length), the cartridge or column can be operated at room pressure under gravity-flow conditions or at high pressures.

From the point of view of automation, the sorption technique can be applied in a continuous or discrete fashion in relation to the analyser or instrument. This is the criterion used to describe the systems commented on below.

### 4.6.1 Off-line sorption systems

This type of system is most commonly used in the collection and preconcentration of toxic substances from the atmosphere by means of withdrawal pumps fitted to small columns located at strategic points and connected to an automatic programmed thermal desorption instrument coupled to a gas chromatograph. A detailed description of these systems is given in Chapter 12.
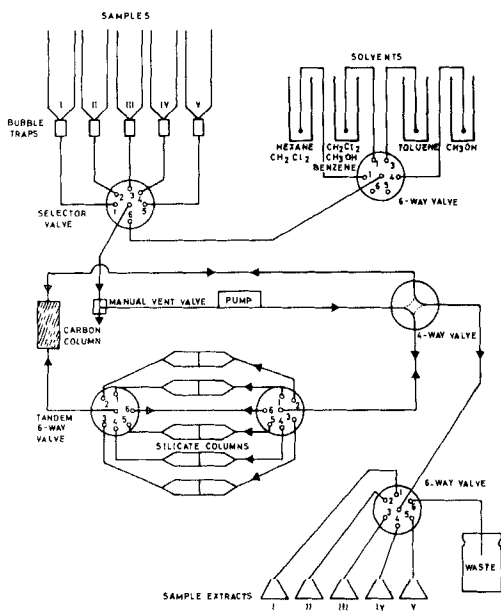


**Fig. 4.12** Automatic on-line sorption system for chemical clean-up used in the determination of TCDD in tissue samples. (Reproduced from [7] with permission of the American Chemical Society).

Lapeza *et al.* [7] recently reported an automatic system (Fig. 4.12) for pretreatment (sorption and preconcentration) of 2,3,7,8-tetrachlorodibenzo-p-dioxin in human adipose tissue for its subsequent determination at the parts per $10^{12}$ level by high-resolution gas chromatography-mass spectrometry. The microprocessor used allows the control of the functioning of the pump and a series of valves (a six-position rotary, a four-way rotary and a tandem six-

position one). The system uses sample extraction (I-V), potassium silicate/sil-
ica and carbon/glass fibre columns. Air bubbles must be kept out of the system
—particularly out of the pump head. At time zero, the pump is started and the
selecting valve is set to receive sample extract from the sample column (I),
which is driven to the silicate and carbon columns. As the program runs, the
silicate column is removed from the flow, the carbon column is washed and then
eluted in the reverse direction and, finally, both columns are regenerated by
multiple solvent washings. Neither the pretreatment of the tissue specimens
nor the introduction of the extracts into the instrument —requiring solvent
changeover— are automated.

a)
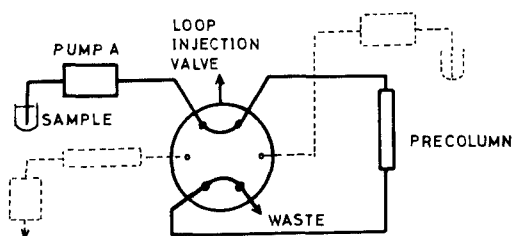


b)
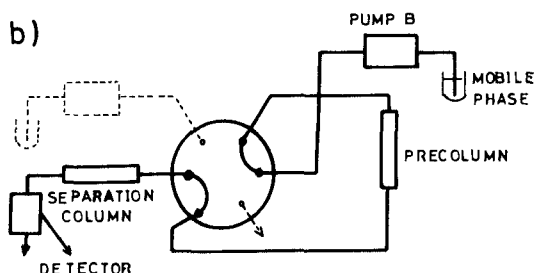


**Fig. 4.13** Scheme of on-line sorption system for preconcentration of
liquid samples prior to introduction into a liquid chromatograph. (A)
Preconcentration stage; (B) elution-determination stage. (Reproduced
from [8] with permission of Elsevier).

### 4.6.2 On-line sorption systems

In this alternative, the liquid eluted from the sorption system —general-
ly a column— is introduced directly into the analyser or instrument [8,9]. A
scheme of the typical functioning of these systems is depicted in Fig. 4.13.
The system operation consists of two stages. In the first, the sample is aspi-

rated by pump A through a valve into the pre-column and then to waste —this allows the sample volume taken to be adjusted according to the analyte concentration. In the second stage, the valve is switched to another position and a high-pressure pump (B in Fig. 4.13) propels the mobile phase through a pre-column into the chromatographic column. The analytes are rapidly released from the pre-column and led to the instrument. This assembly can be made more complex by using several pre-columns and valves. The potential of this operational is discussed in greater detail in Chapter12.

There are several commercially available instruments using sorption systems for sample pretreatment. A representative example of those working in a continuous fashion is the Varian AASP (Advanced Automated Sample Processor) [10]. This uses cassettes of ten sorbent cartridges, each of which is packed with 40-50 mg of a particular bonded silica gel. It features three essential differences from typical manual systems, namely the use of lower sample and solvent volumes, the utilization of pressure rather than vacuum and the fact that the liquid emerging from the cartridge is completely eluted to the HPLC system, thereby increasing the sensitivity. Analytical Biochemistry Laboratories (ABC) markets a module (GPC 1002 A) for automated clean-up of residues in extracts by use of a gel-permeation column.

## 4.7 ION EXCHANGE

The use of ion-exchange columns for trace preconcentration and interference removal is a mode of the sorption processes described above. The chief difficulty encountered in using ion-exchange column for these purposes is the low selectivity of the usual cation and anion exchange resins, particularly when solutions with high saline contents are involved (e.g. sea water matrices). This has fostered the use of more selective resins such as those containing chelating iminoacetic groups or reagents such as hydroxyquinoline or thiols immobilized in organic or inorganic matrices.

Ruzicka, the head of one of the pioneering teams in FIA, designed various assemblies for the preconcentration of traces of metal ions prior to their determination by atomic absorption or ICP spectroscopy in a variety of liquid matrices by use of small columns (5 mm x 2 mm I.D.) packed with the chelating resin Chelex-100 (50-100 mesh). The most interesting of such assemblies [22] are shown in Fig. 4.14. The first assembly (Fig. 4.14a), the simplest of all, features a single line with two serial injection valves ($I_1$ for insertion of sample and $I_2$ for introduction of the eluent) prior to the column on which the analytes are retained. First the sea water sample is injected and driven to

the column, where the analytes are retained. Then, 2M HNO₃ is injected to elute the analytes, which are led to the detector. The column is continuously regenerated by passing the carrier with ammonium acetate buffer through its bed. This assembly poses some problems arising from the lack of homogenization between the carrier and the sample, and the uneven swelling of the resin beads in passing from the ammonium to the acid form.

The configuration depicted in Fig. 4.14b was designed to overcome the shortcomings of the previous type. The sample is homogenized by injecting it into an aqueous carrier merging with a buffer stream. Uneven swelling of the resin is avoided by passing the eluent in the opposite direction to the sample. The change of the direction of flow is achieved with the aid of an additional valve —the other two valves are placed on both sides of the column. In the preconcentration position (b.1) the extra valve drives the eluent stream to the detector as the injected sample passes through the column and goes to waste. In the elution position (b.2), the valves are switched to establish two flows in opposite directions: that of the sample is led to waste, whereas that of the eluent is passed in the opposite direction through the column and then driven to the detector.

The most automated of these systems is shown in Fig. 4.14c. It includes a valve replacing the two used in the previous configuration and a timer ensuring the intermittent functioning of the two pumps. During the preconcentration cycle, pump $P_1$ propels up two three lines (two with water and another with buffer); the sample pH is adjusted and one of the water streams is sent to the detector. The stream emerging from the column is also controlled by pump $P_1$. In the elution stage, pump $P_1$ is stopped as $P_2$ is started. The HNO₃ stream is forced to circulate upstream through the column and is driven to the detector —it cannot go to waste as pump $P_1$ is stopped. Meanwhile, the next sample is aspirated into the injection valve loop.

These assemblies have the shortcoming that they cannot be used with large sample volumes, preconcentration of which could be inefficient. To overcome this drawback the FIA configuration depicted in Fig. 4.15 [12], based on the use of two columns packed with Chelex-100 and positioned parallel to each other was developed. The configuration consists of two pumps and two injection units which function simply to change the direction of the flows —they have no volume-meter loops. One of the units is connected via eight lines with two loops containing the two columns and the other has two connections only. Two samples, A and B, mixed with their respective buffers, can be manipulated in a virtually simultaneous manner. In position 1, the streams pass sequentially through the columns and are sent to waste. A water stream is continuously circulated through the detector (ICP) and the eluent stream is driven to waste. Elution is performed in two sequential stages: in the first (2B in Fig. 4.15),
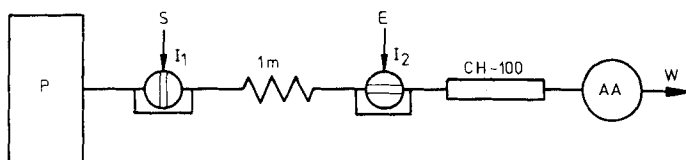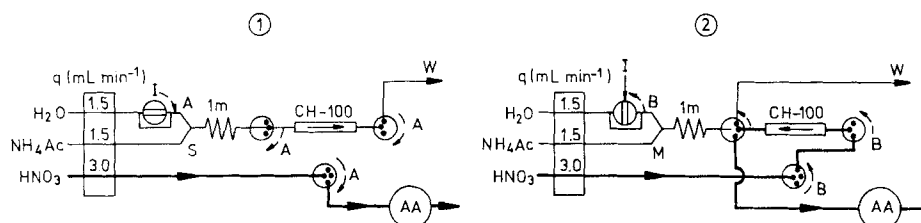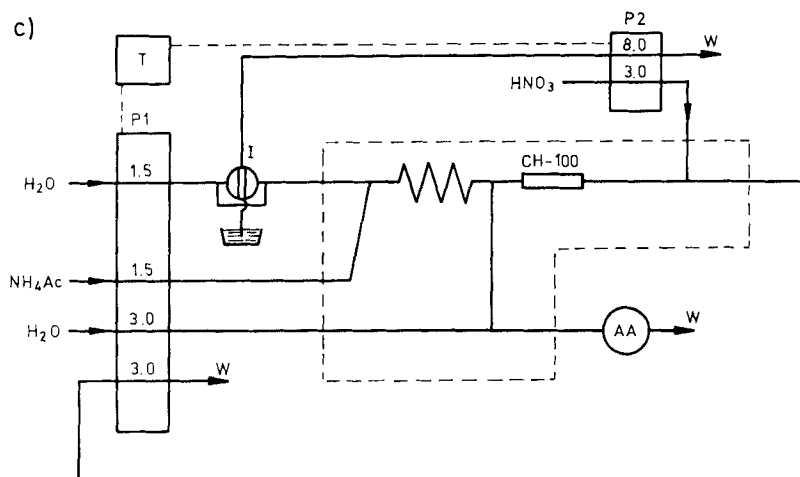
**Fig. 4.14** Flow-injection assemblies for ion-exchange preconcentration of metal traces prior to introduction into an atomic absorption spectrometer. (A) Single-line manifold; (B) three-valve system for upstream elution; (C) automated assembly with intermittent pumping. (Reproduced from [11] with permission of the Royal Society of Chemistry).

the valves are switched so that the eluent flow may pass through one of the columns —that corresponding to sample B— into the detector. As the smaller valve is switched to position 2A, the eluent stream passes through the column for sample A and on to the detector. A further advantage of this system is its increased sampling frequency, a result of the possibility of treating two samples simultaneously in the slower stage (preconcentration).
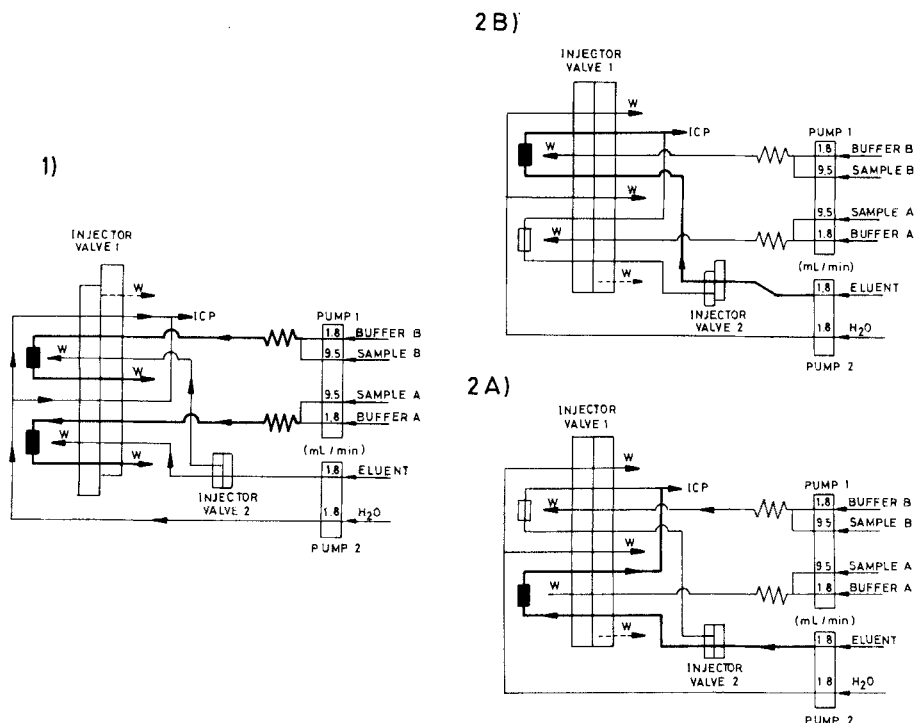


Fig. 4.15 Continuous assembly for simultaneous preconcentration of two samples (A and B) by use of two parallel ion-exchange columns. (1) Preconcentration stage. (2) Sequential elution stage. (Reproduced from [12] with permission of the American Chemical Society).
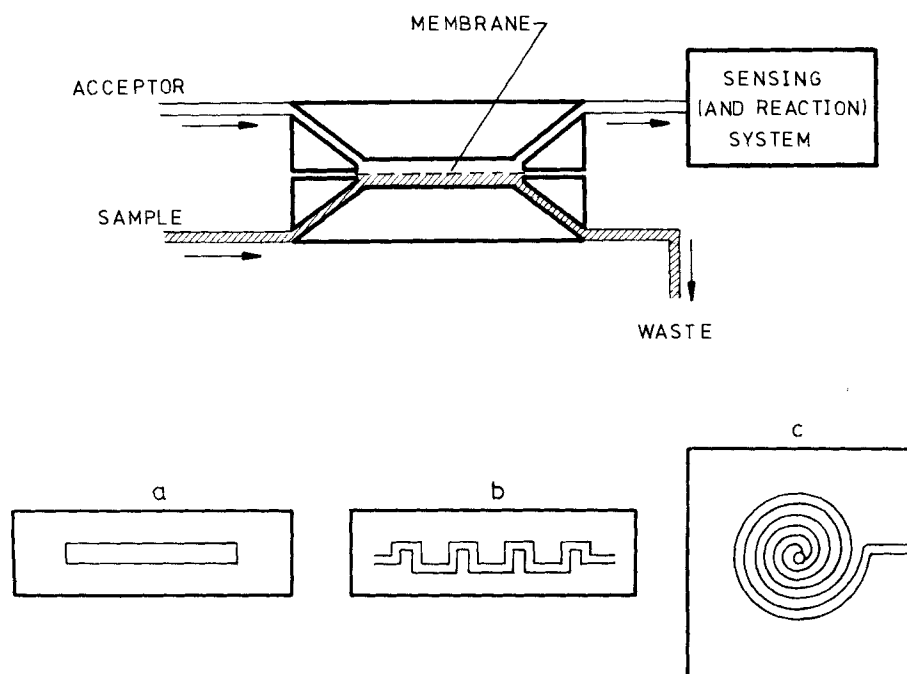
## 4.8 MEMBRANE SEPARATION

Separations based on the use of membranes have gained significance in the context of Analytical Chemistry. The large variety of commercially available membranes facilitates their use for a variety of applications. Their functioning is based on the selective passage of a substance or a group of substances with given characteristics.

Membrane separation is generally implemented in a continuous fashion. The essential components of a continuous module are shown in Fig. 4.16. A carrier solution containing the sample —to which reagents facilitating the separation

can be previously added— is led into a minichamber whose central zone is oc-
cupied by the membrane, which divides the chamber into two symmetrical parts.
On one side of the membrane circulates the carrier stream and on the other
side circulates, in the same direction, a suitable reagent facilitating the
dissolution or incorporation of the substances separated across the membrane.
This module usually consists of two blocks made of various materials into
which the same conduits are engraved, and the minichamber —generally a paral-
lelepiped and less often in a tortuous or spiral shape to increase the separa-
ting efficiency through increased contact surface— the two halves of which
are tightly pressed on the membrane to avoid losses.



**Fig. 4.16** Schematic diagram of continuous membrane separator. (a)
Parallelepiped, (b) tortuous and (c) spiral separation chamber.

Depending on the nature of the substances to be separated, one can distin-
guish between two chief types of continuous membrane separation:

(a) *Dialysis*, in which the separation is generally based on the differ-
ences in molecular weight between the sample components. Thus, small molecules
and ions pass through the membrane pores, whereas bulkier species remain in
the carrier. This type of separation is widely used in the determination of
species with low molecular weights in biological fluids.

(b) *Gas diffusion*, which involves the use of gas-permable membranes. Gas species can be formed in the sample stream through a variety of reactions, the commonest of which are induced by pH changes to form volatile acids (HCN, $CO_2$, $SO_2$) and bases ($NH_3$). There is also the possibility of determining dissolved gases such as $O_2$, $O_3$, $Cl_2$ and $ClO_2$. The acceptor solution must contain reagents that interact with the diffused gases to ensure their rapid retention. Dialysers are frequently used with continuous analysers, particularly in clinical applications, a comprehensive description of which is beyond the scope of this book. Only two recently reported examples of the application of this technique in two different areas are commented on below.
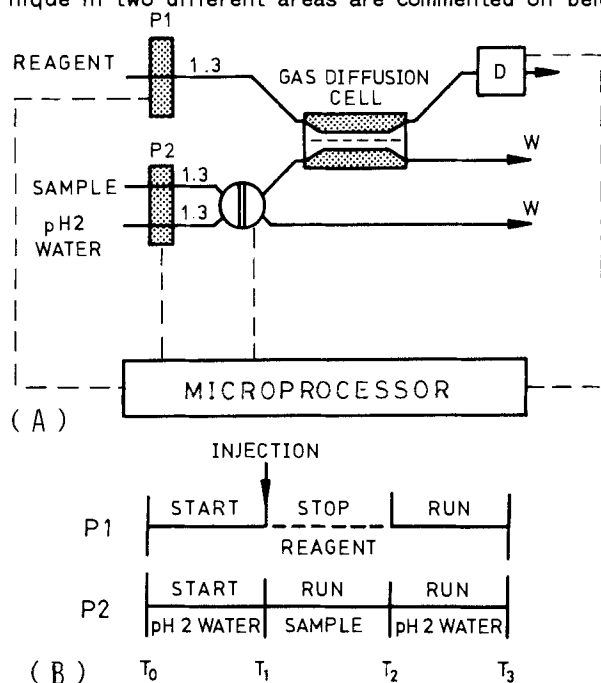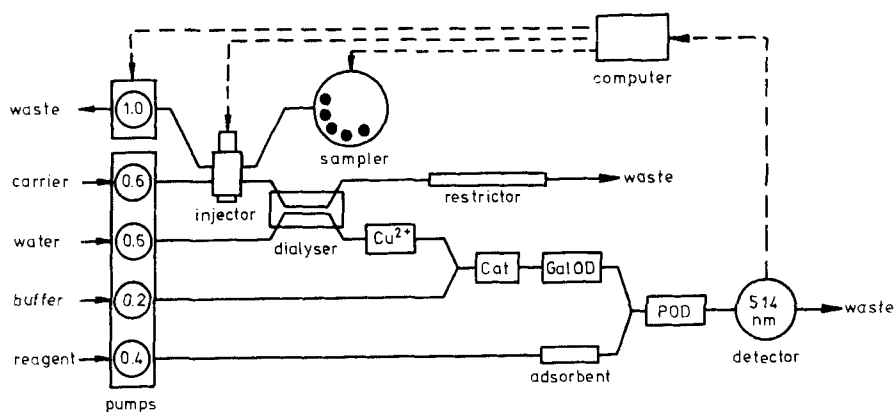


**Fig. 4.17** (A) Automatic determination of residual ozone in water by liquid diffusion with two pumps ($P_1$ and $P_2$) working intermittently under the control of a computer. (B) Functioning of the peristaltic pumps and the injection valve. (Reproduced from [13] with permission of the American Chemical Society).

Figure 4.17 shows the scheme of the system designed by Straka *et al.* for the determination of residual aqueous ozone [13]. The gas-diffusion cell (a Chemifold V from Tecator), with a 75 x 2 $mm^2$ area, is fitted with either a dimethylsilicone or a 0.45 μm pore size Teflon membrane. A valve allows the introduction of either a continuous sample stream or a carrier of pH 2. The ac-

ceptor stream contains a dissolved dye, potassium indigotrisulphonate, which rapidly becomes colourless upon reaction with ozone. A continuous photometric detector with a flow-cell monitors the absorbance decrease, proportional to the ozone concentration. The gas-diffusion separation can be performed with a specific period of stopped flow to enable more of the gaseous analyte to permeate through the membrane. Figure 4.17 also shows the diagram of a peristaltic pump and the injection programming carried out by means of the built- in microprocessor of the Tecator Flow Injection Analyzer used. The pump propelling the carrier and sample ($P_2$) works in a non-stop fashion. At the beginning of the sequence ($T_0$), pumps $P_1$ and $P_2$ function as a stream of water at pH 2 passes below the reagent stream. At the time of sampling ($T_1$), the selecting valve switches the flow from water of pH 2 to the ozone sample. Simultaneously, pump $P_1$ stops for the desired interval (10, 20 or 30 s), $T_2-T_1$. During this stopped-flow period, ozone permeates through the membrane. After the sampling period, pump $P_1$ is started again and pumps the decolorized reagent to the detector while the selecting valve is switched so that water of pH 2 is again passed below the reagent. This also results in preconcentration. Detection limits of *0.03 mg/L* of ozone are possible, with sensitivities and linear ranges comparable to those of the manual counterpart of the method. The selectivity is also significantly increased and the interference of chlorine is reduced to 0.008 mg/L of apparent ozone for each part per million of chlorine. The sample frequency is 65 $h^{-1}$.



**Fig. 4.18** Automatic enzymatic determination of D-galactose in serum in a continuous FIA system with liquid dialysis for the removal of interfering macromolecules. Reactors: $Cu^{2+}$, Bond-Elut-NH$_2$-Cu; Cat, catalase; GalOD, galactose oxidase; POD, peroxidase. Absorbent: arylamino porous glass. (Reproduced from [14] with permission of Elsevier).

An interesting example of continuous dialysis in enzymatic systems applied to clinical samples is the determination of D-galactose in serum via a series of coupled enzymatic reactions [14]. Figure 4.18 shows the dedicated computerized automatic assembly constructed for this purpose. The samples are aspirated sequentially into the loop of an injection valve. The sample inserted in the carrier is driven to the dialysis unit, after which the carrier stream is sent to waste through a restrictor intended to match the back-pressure on the detector side of the dialyser. An acceptor stream leads the dialysed serum components to (a) a Bond-Elut-NH₂-Cu column for removal of interferents, (b) a catalase reactor for removal of $H_2O_2$ generated in reactions taking place in the copper reactor and (c) a galactose oxidase reactor which produces $H_2O_2$ from the analyte. The flow is merged with a reagent stream, which gives rise to an oxidative coupling reaction in the peroxidase reactor. The final detection is performed spectrophotometrically at 514 nm. The dialyser used consists of two equal Perspex halves with cut channels, clamped together over a 0.025 mm thick cellulose acetate membrane. The dialyser channels can be filled with solid glass beads (150-250 μm in diameter) to reduce dispersion. The linear range achieved is from 10 μM to 14 mM D-galactose, and the recovery from spiked serum samples at low analyte levels is close to 100%.
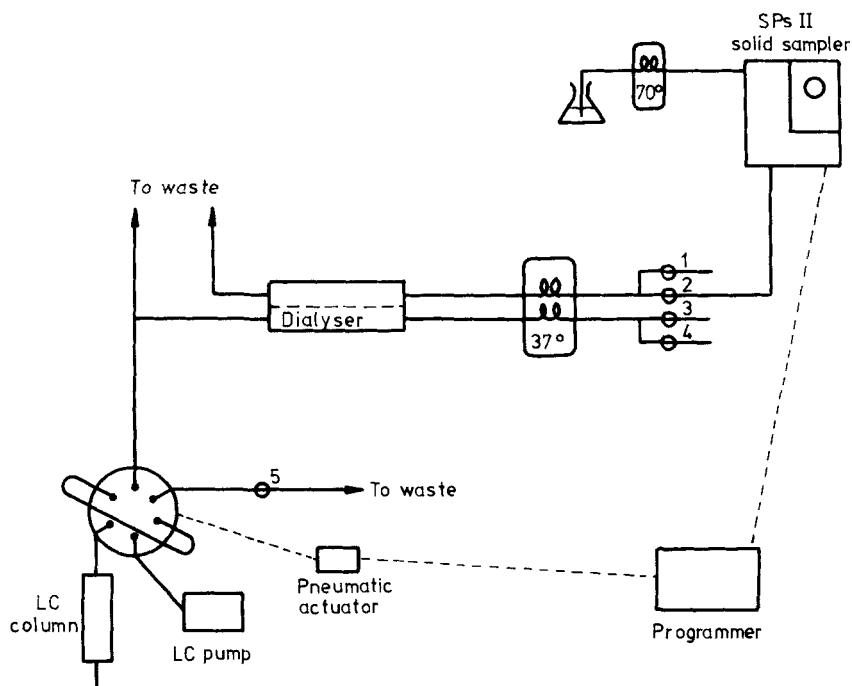
## 4.9 SOLID-LIQUID EXTRACTION

This selective lixiviation or dissolution operation can be performed automatically by the Technicon SOLIDprep II module. The undissolved solid gathers in the bottom of the extraction vessel, so that the aspirating pipette readily withdraws the supernatant liquid.

A representative example of this type of assembly is shown in Fig. 4.19, which was designed by Coverly [15] for the determination of water-soluble vitamins (nicotinamide, thiamine, pyridoxine, riboflavin) in pharmaceuticals by reversed-phase HPLC, at a sampling rate of 10 samples/h. The extractant solution, a phosphate buffer with heptenesulphonate and triethylamine previously heated to 70°C, is introduced into the SOLIDprep II, where first mild stirring (500 rpm) and subsequently rapid stirring (2900 rpm) facilitate extraction of the water-soluble fraction. The aspirated extract is previously passed through a dialyser. The solution collecting the vitamins separated across the membrane fills the loop of the chromatographic injection valve. A programmer synchronizes the discontinuous functioning of the solid-liquid extraction unit and the introduction into the chromatograph.

Technicon designed another system of this type for the determination of hexylresorcinol in pharmaceutical tablets by simultaneous dissolution and ex-

traction [16]. Each of the twenty cups of the solid sampler contains five tab-
lets which are mechanically poured into a homogenizer. This receives preset
volumes of distilled water and chloroform and is stirred for a given time to
increase contact between the three phases originally present. Once this opera-
tion has terminated, the supernatant is aspirated into a fraction collector
without stopping the stirring. This configuration requires no phase separation
as phases are decanted in each vial. The fraction collector is connected on-
line with a gas chromatograph. Sampling is effected by a suction system; the
aspirating tip dips into each vial until it finds the organic heavier phase,
an aliquot of which is taken. The configuration uses two turntables, one con-
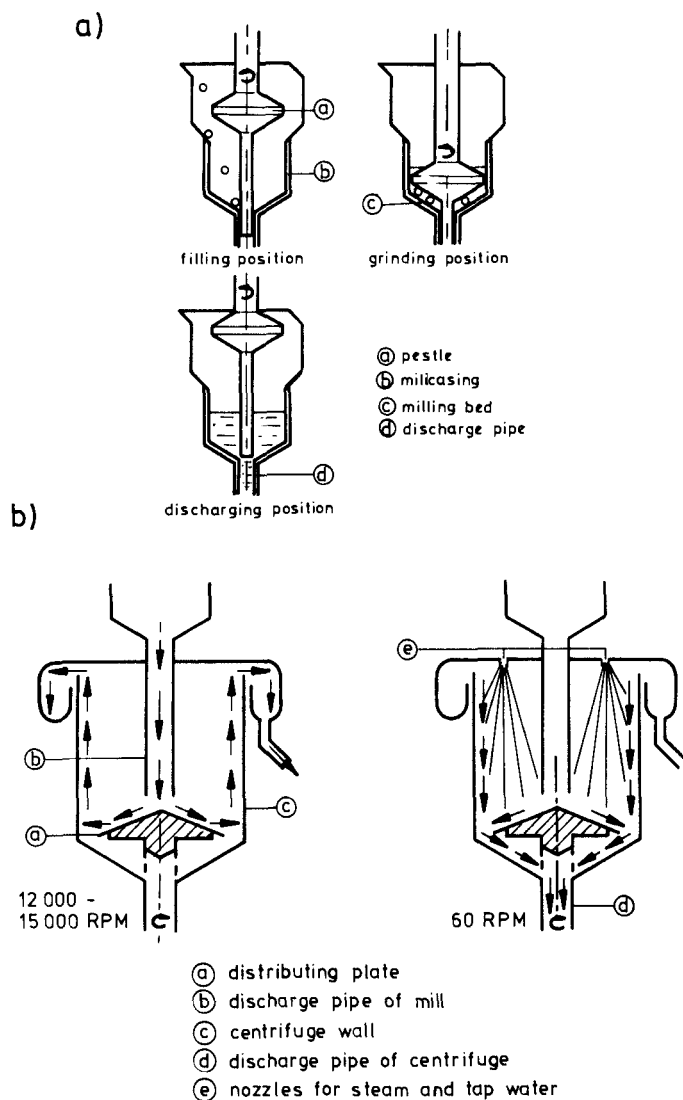taining the solid samples and the other holding the corresponding extracts.



**Fig. 4.19** Continuous solid-liquid extraction assembly for the de-
termination of water-soluble vitamins in pharmaceuticals as a prelim-
inary step to their introduction into a liquid chromatograph. (Repro-
duced from [15] with permission of Francis & Taylor Ltd.).

The problem of the state of aggregation of the sample is a major considera-
tion in the automation of the preliminary stages of the analytical process;
however, it has been tackled only rarely, probably because of the technical
difficulties involved. Problems such as the potentially different compositions

of grains of different size, the varying dissolution/extraction time depending on the state of aggregation, etc. are not generally dealt with in a systematic way. One of the few exceptions in this respect is the system reported by Bartels *et al.* [17], a microprocessor-controlled assembly consisting of the following parts (Fig. 4.20):

a)



filling position          grinding position

ⓐ pestle
ⓑ millcasing
ⓒ milling bed
ⓓ discharge pipe

discharging position

b)



12 000 –
15 000 RPM                                    60 RPM

ⓐ distributing plate
ⓑ discharge pipe of mill
ⓒ centrifuge wall
ⓓ discharge pipe of centrifuge
ⓔ nozzles for steam and tap water

**Fig. 4.20** (A) Combined griding and solid–liquid extraction system for solid samples. (B) Subsequent phase separation by high-speed centrifugation. (Reproduced from [17] with permission of Francis & Taylor Ltd.).

(1) Simultaneous grinding and extraction unit. The samples (pellets, grains, tablets, capsules, suppositories, etc.) are introduced together with the solvent or a mixture of solvents in the filling position, where the rotary mortar is in an intermediate position. In the grinding position, the mortar is lowered, rotated and moved upside down to grind the samples and bring the solid into contact with the extractant liquid. After a preset time, the mortar is raised (discharging position) and the suspension is passed on to the next unit.

(2) Centrifuging unit, which features a central conduit through which the suspension is passed. A distributing plate rotating at a speed of 12,000–15,000 rpm receives the suspension frontally and throws it abruptly at the walls. The liquid creeps upwards on the centrifuge wall, gathers in an external channel and is removed for subsequent determination, filling the original sample cup. A series of nozzles located at the top of the centrifuge allow it to be washed between samples. The distribution plate rotates slowly so that the clean solutions may wash over it and be removed through the discharging tube. The final residue can be either discarded or redissolved in a suitable solvent and collected in a second sample cup in the transport mechanism and be subsequently analysed.
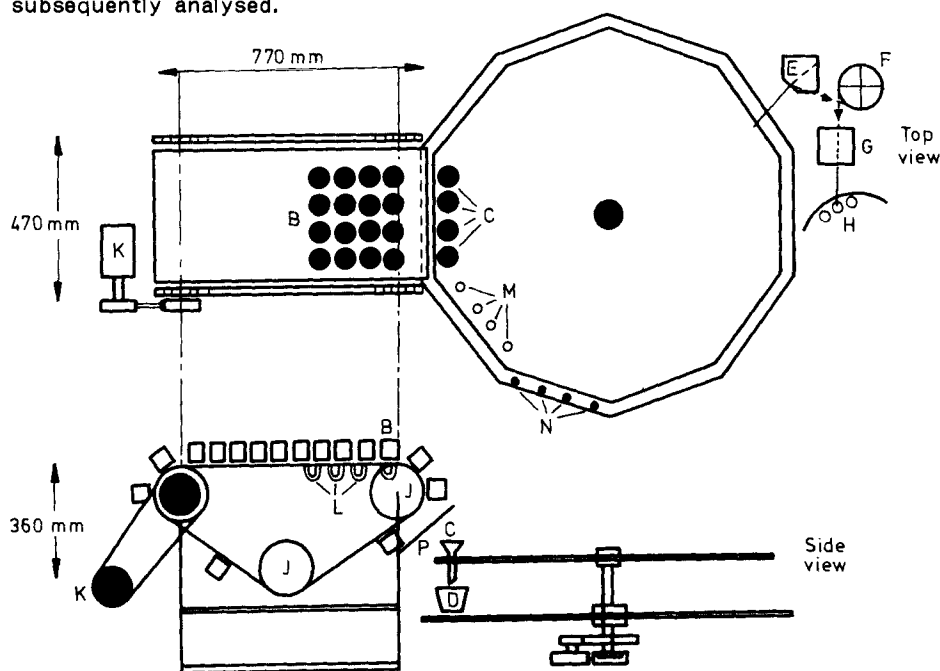


**Fig. 4.21** Automated discrete solid–liquid extraction system for determination of mineral nitrogen in soils. (Reproduced from [18] with permission of the Royal Society of Chemistry).

Marsh *et al.* [18] reported an interesting automatic configuration for the determination of mineral nitrogen in soils without human intervention (Fig. 4.21). The samples are weighed in special vessels. An adequate extractant volume is added by means of an automatic, electronically controlled, home-made system based on a basic beam balance. After addition of the reagent, a magnetic stirrer is placed in each beaker and the beakers are fitted into cups of the conveyor on the sample preparation unit (B). The beakers are carried over four rows of revolving magnets to give a total of 48-min stirring and stand stirred for a further 12 min, after which they are tipped into filter-funnels (C) around the periphery of a ten-sided Perspex turntable. The soil extract, filtered through a Whatman no. 1 filter paper, is collected in a beaker (D) fitted to a similar turntable lying below the filter table. The tables rotate on a common spindle until the sample reaches a sample pick-up arm (E) and is drawn up via a peristaltic pump (F) and automatically diluted when needed and dispensed via another arm (G) into the cups of an AutoAnalyzer tray (H).

Hormann *et al.* developed a much more complex assembly for the extraction, clean-up and gas chromatographic determination of triazine herbicides in soil [19].

## 4.10 LIQUID–LIQUID EXTRACTION

The automation of liquid–liquid extraction processes is of great interest on account of the relevance of this technique to the sample treatment and of the technical complexity of such processes when carried out manually.

The reduction of human intervention in this preliminary operation can be achieved in a variety of ways:

(a) By applying automatic batch methodologies in which both phases are brought into contact in a vessel to form an emulsion.

(b) By the Craig counter-current extraction.

(c) By circulating the volatile phase through the other by means of a distillation-condensation system.

(d) By circulating alternate segments of both phases along a flow system.

(e) By circulating a mobile phase over a stationary phase retained in one of the various possible ways (liquid–liquid partitioning chromatographic configurations).

A detailed description of all these alternatives is beyond the scope of this book, so only representative examples of two modes, namely batch and continuous, are discussed here.

### 4.10.1 Batch liquid–liquid extraction

This is uncommon as it is resorted to when long equilibration times are

involved as a result of kinetic effects. Figures 4.22 and 4.23 illustrate two automation possibilities differing in the way in which the final phase separation is effected once the emulsion has been destroyed —generally by agitation with a paddle rod. In the assembly depicted in Fig. 4.22, a microcomputer controls all the operations involved, namely:

(a) Addition of samples by aspiration of a preset volume or collection of a given amount quantized by any other means.

(b) Addition of controlled volumes of the reagent and of the other phase —generally the organic phase.

(c) Controlled stirring for a given period at a preset speed.

(d) Collection of the phase of interest, which can be effected in various ways. If it is the heavier phase, it is drained by a valve governed by a continuous-flow sensor based on refractive index measurements reflecting the passage of the interface and controlled by the computer. If it is the lighter phase, an aspiration probe positioned at the appropriate height or a gas pressure-based system is more than adequate —this alternative is also suitable for separation of the heavier phase.

(e) Clean-up of the extraction vessel by means of an addition, stirring and drainage system which renders it ready to receive a fresh sample.
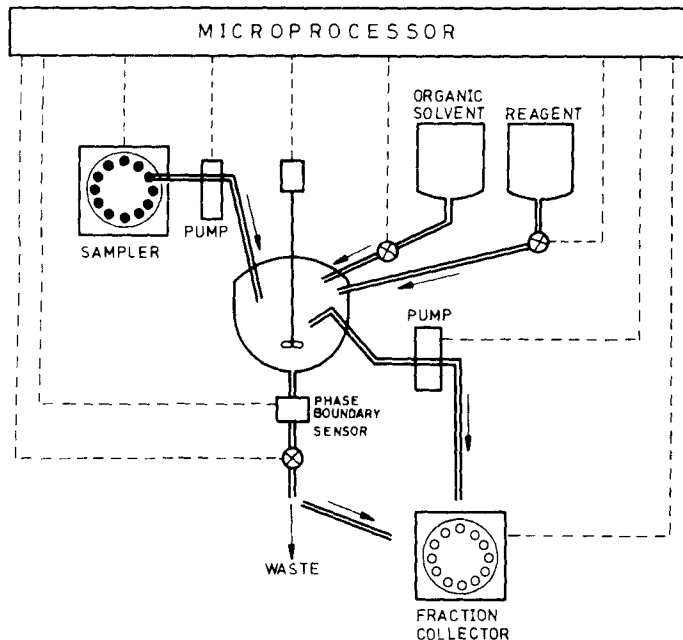


**Fig. 4.22** Scheme of computer-controlled batch liquid-liquid extraction process.
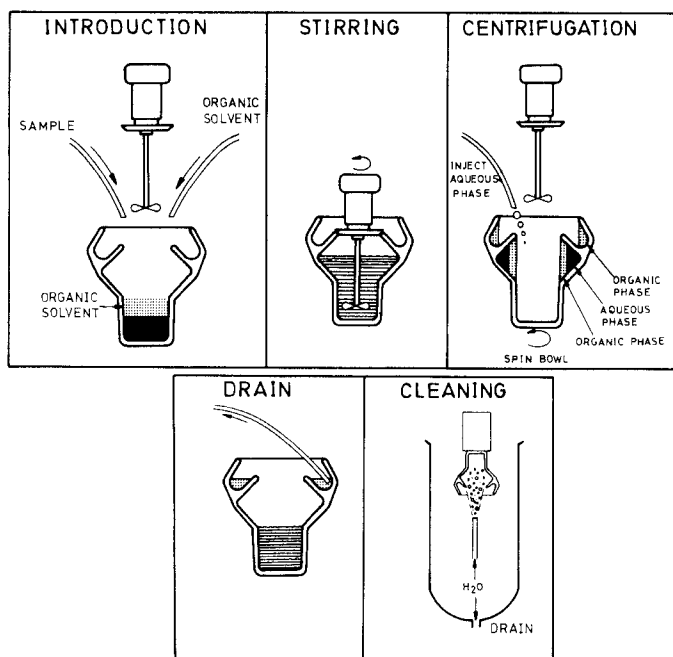
**Fig. 4.23** Automatic batch liquid-liquid extraction configuration based on phase separation by centrifugation. (Reproduced from [20] with permission of the Association of Official Analytical Chemists).

Figure 4.23 illustrates another alternative for the separation of both phases once the batch liquid-liquid extraction has been finished. Formerly conceived for solid-liquid extractions, this complex mechanical assembly [20] consists of two automatic burettes for addition of the two phases, the extractor —moveable in various fashions— and a vertically moving paddle stirrer. The extraction vessel rotates at a high speed, which promotes phase separation, as shown in the figure. The lighter phase creeps up the walls and passes to an upper receptacle —the separation is facilitated by adding more aqueous phase. Once separation is complete, an aspiration probe withdraws the organic phase. Finally, a mechanical system turns the vessel over for cleaning.

### 4.10.2 Continuous liquid-liquid extraction

Of the various possible ways in which this alternative can be implemented, that based on the establishment of a segmented flow is without doubt the most advantageous from the point of view of automation and scope of application [21,22]. Figure 4.24 depicts the scheme of the essential components of a continuous liquid-liquid extractor, namely:

(1) The *solvent segmenter*, which acts as a merging point for the two initial streams of the two phases converging on it. It is chiefly intended to obtain alternate, regular segments of both immiscible liquids entering the extraction coil. It should be designed so that the 'length' of the segments can be conveniently checked. The commonest type of segmentor consists of a tube with three openings [23]. The aqueous phase enters through a glass capillary and the organic phase through a platinum capillary perpendicular to the former. Two adjustable concentric Teflon tubes shut off the tube at the other opening in the same direction as the aqueous phase. The height of the inner tube is adjustable. The length of the segments emerging from this tube depends on (a) the aforesaid height, (b) the inner volume of the mixing chamber and (c) the flow-rate of both phases or their ratio ($q_o/q_a$), which is equivalent to the volume ratio in batch liquid–liquid extraction processes. The commonest situation involves identical segments about 5 mm in length.

(2) The *extraction coil*, which receives the segmented flow and holds it for a preset time and serves as a receptacle for the transfer of matter taking place between both phases. There are two general possibilities depending on the type of material of which the coil is made. If made of Teflon, the organic phase wets its walls and the aqueous phase is in the form of bubbles. The flow patterns in coiled solvent segmenters and the direction of the solute transfer are shown in Fig. 4.24. In addition to gravitational mixing, effective mass transfer is the result of the plug flow in the opposite direction of the segments and of the secondary flow patterns formed in a coiled tube. Hence, tubes of small bore (0.5–1 mm) make good extraction coils.

(3) The *phase separator* receives the segmented flow from the coil and continuously splits it into two separate streams of the two phases. The separation is rarely quantitative and usually ranges between 80 and 95%. However, the phase in which the determination is to be carried out should be kept completely free from the other so that no parasitic signal is originated on passage through the detector. Of the different technical alternatives, T-shaped separators use gravity with or without a sort of phase guide made of a material wetted by one phase but not by the other [23]. Figure 4.24 shows a separator of this type which features a piece of Teflon stuck to the inside which aids the separation of the lighter organic phase up to the exit. The most advantageous devices of this kind, though, are membrane separators, based on the selective permeability of a microporous membrane (0.7–0.9 μm pore diameter) towards the phase which wets the membrane material —usually Teflon. The organic phase crossing it is completely free from aqueous phase.
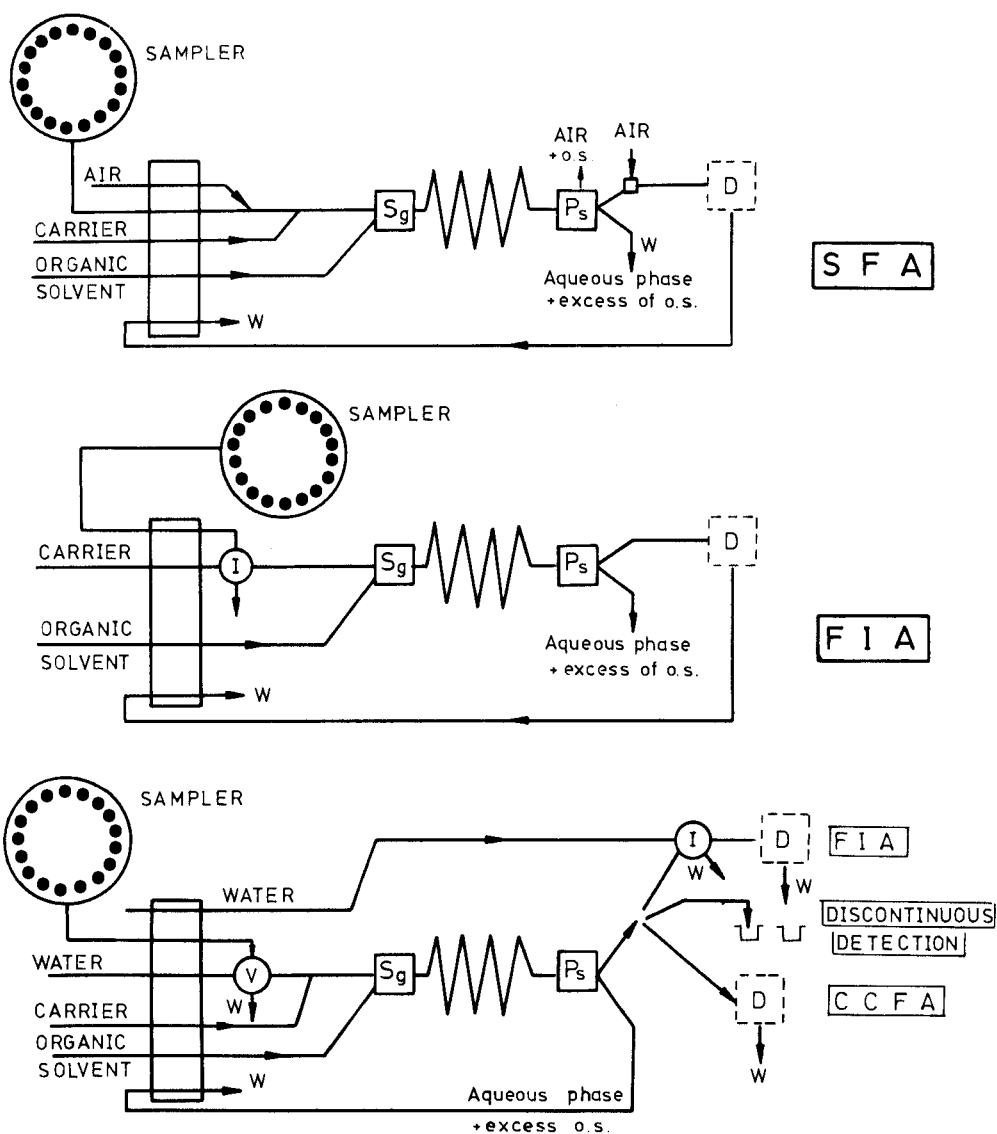
Figure 4.25 shows schematically the manifolds most frequently employed with automatic continuous liquid–liquid extraction. All of them have the following
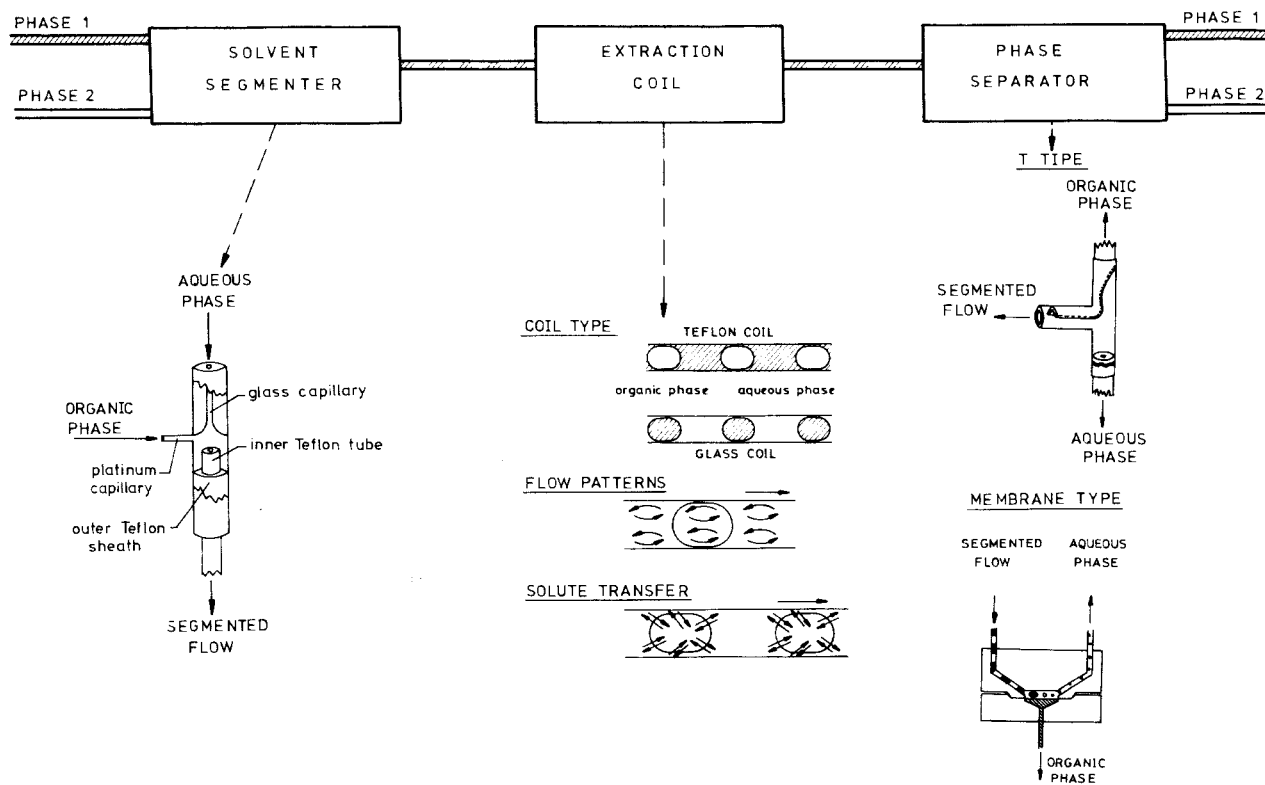
**Fig. 4.24** Components of a continuous liquid-liquid extractor based on flow segmentation. (Reproduced from [21] with permission of Ellis Horwood).

**Fig. 4.25** Different ways of coupling continuous liquid-liquid extraction to air-segmented (SFA), flow-injection (FIA) and completely continuous flow analysers (CCFA).

common elements: a sampler, a peristaltic pump, two channels (one for a car-
rier containing a reagent or not and another for the organic solvent), a sol-
vent segmenter, an extraction coil and a phase separator. The essential dif-
ferences between SFA and FIA configurations are (a) the air bubbles inserted at
two points prior to merging with the carrier and after separation of the two
phases and (b) the manner in which samples are inserted (aspirated in SFA and
injected in FIA). In the configuration in Fig. 4.25c, the sample is aspirated
through a two-way valve and there are three possibilities, depending on the
type of detection system used: (a) CCFA, when the analyte phase flows directly
to the detector flow-cell; (b) FIA, when the analyte phase fills the loop of an
injection valve and the phase plug is carried to the detector unit; (c) dis-
crete detection, when the phase containing the analyte is collected in several
cups and detection is performed individually in a fraction collector.

The continuous liquid–liquid extraction carried out by FIA systems offers
clear advantages over those implemented on SFA manifolds, namely: (a) lower
sample, reagent and organic solvent consumption, (b) faster determinations, (c)
simpler design, (d) greater reproducibility and (e) lower cost. The earliest
FIA methods associated with liquid–liquid extraction were proposed simultane-
ously by Karlberg and Thelander [24] and Bergamin *et al.* [25] in 1978. Figure
4.26 illustrates two examples representing the numerous systems of this type
described to date. Shelly *et al.* [23,26] developed a continuous FIA system with
multi-extraction. That shown in Fig. 4.26a was conceived for interference
removal in the fluorimetric multi-determination of carcinogenic polynuclear
aromatic hydrocarbons (PAHs). A crude oil/ash sample residue (500 µL) dissolved
in cyclohexane is injected into a stream of the same organic solvent that mer-
ges with a DMSO stream in the first solvent segmenter. After passing through a
glass extraction coil, the DMSO phase is mixed with a water stream in a cooling
coil. In the second solvent segmenter, a fresh stream of cyclohexane is seg-
mented with the aqueous DMSO phase. After passage through a Teflon extraction
coil, the organic phase is carried through the flow-cell of a video fluori-
meter. The optimized system has a sample recovery similar to that of an iden-
tical manual procedure and a 1.5% relative standard deviation between injec-
tions. The sampling rate is 12 h$^{-1}$.

The authors' team recently developed a configuration for the indirect
atomic absorption spectroscopic determination of anionic surfactants in waste
water by FIA/continuous liquid–liquid extraction (Fig. 4.26b). The sample is
continuously introduced into the system via a peristaltic pump and merged with
a carrier containing the cationic chelate 1,10–phenanthroline–Cu(II) at pH 4.5,
which forms an ion pair with the detergent to be analysed. This last is intro-
duced into the continuous extractor, where it is transferred to the organic

a)



b)
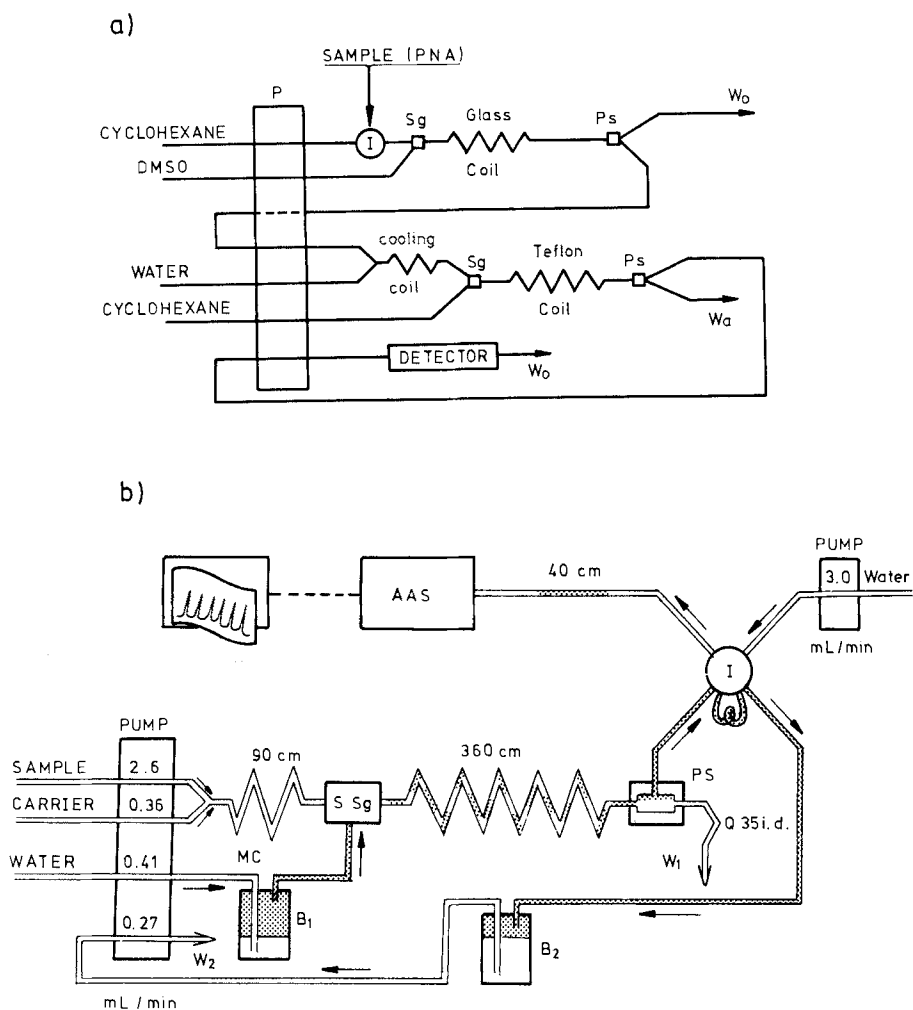


**Fig. 4.26** FIA configurations with continuous liquid-liquid extraction. (a) Multi-extraction system for the fluorimetric determination of poly-nuclear aromatic hydrocarbons; (b) assembly for determination of anionic surfactants in waste water. (Reproduced from [26] and [27] with permission of the American Chemical Society).

phase (MIBK). The organic phase containing the ion-pair and emerging from the membrane separator fills the loop of an injection valve. An aqueous carrier propelled by another pump sweeps the sample plug to the atomic absorption instrument measuring the concentration of extracted copper, proportional to the initial concentration of the anionic surfactant in the water sample. The organic phase streams are established by displacement bottles to avoid their passage through the tubes of the peristaltic pump. The flexible tubes suitable for this purpose have relatively short useful lifetimes. This determination of anionic surfactants in the range 0.1–7.0 µg/mL in waste water offer clears advantages over its manual counterpart, namely (a) higher sampling frequency (45 h$^{-1}$) —the manual method requires two successive extractions—, (b) higher surfactant trace recoveries, (c) better precision and (d) considerably higher selectivity.

The incorporation of continuous extraction systems coupled on line to liquid chromatographs is of great potential and has received especial attention in the last few years. A distinction should be made depending on whether pre- or post-column modules are involved, i.e. according to whether the liquid-liquid extraction precedes or follows the chromatographic process. Figure 4.27 illustrates both alternatives with two representative examples of the numerous systems reported so far.

Figure 4.27a shows the automatic system developed by Dolan *et al.* [28] for the determination of fat-soluble vitamins (A, D and E) in pharmaceutical tablets. It consists of an SFA extraction module located prior to an HPLC system with a UV detector (254 nm). The main elements of this automatic assembly are:

(a) An automated sample preparation unit (SOLIDprep II from Technicon) which receives the tablet from the sample tray, homogenizes it and places it in an ethanol-water mixture.

(b) An air-segmented continuous extraction system that aspirates an aliquot of sample and in which the vitamins are partitioned into hexane to enhance the concentration by a factor of about 4.5. A stream of 5% NaCl is circulated through the centre of the extraction coil to remove water-soluble interferents. After passing through the phase separator unit, the debubbled sample is drawn into a sample loop for automated injection into an HPLC system via a valve. A microprocessor controls the automated solid unit, the injection valve and the UV detector. Injection occurs 7.5 min after the start of the homogenization cycle.

Continuous extraction systems are used after chromatographic separations for two main purposes. One of them is to act as reaction modules (post-column extraction reactors) to facilitate continuous detection via the reaction of the eluted analytes. This option is especially useful for slow analytical reac-
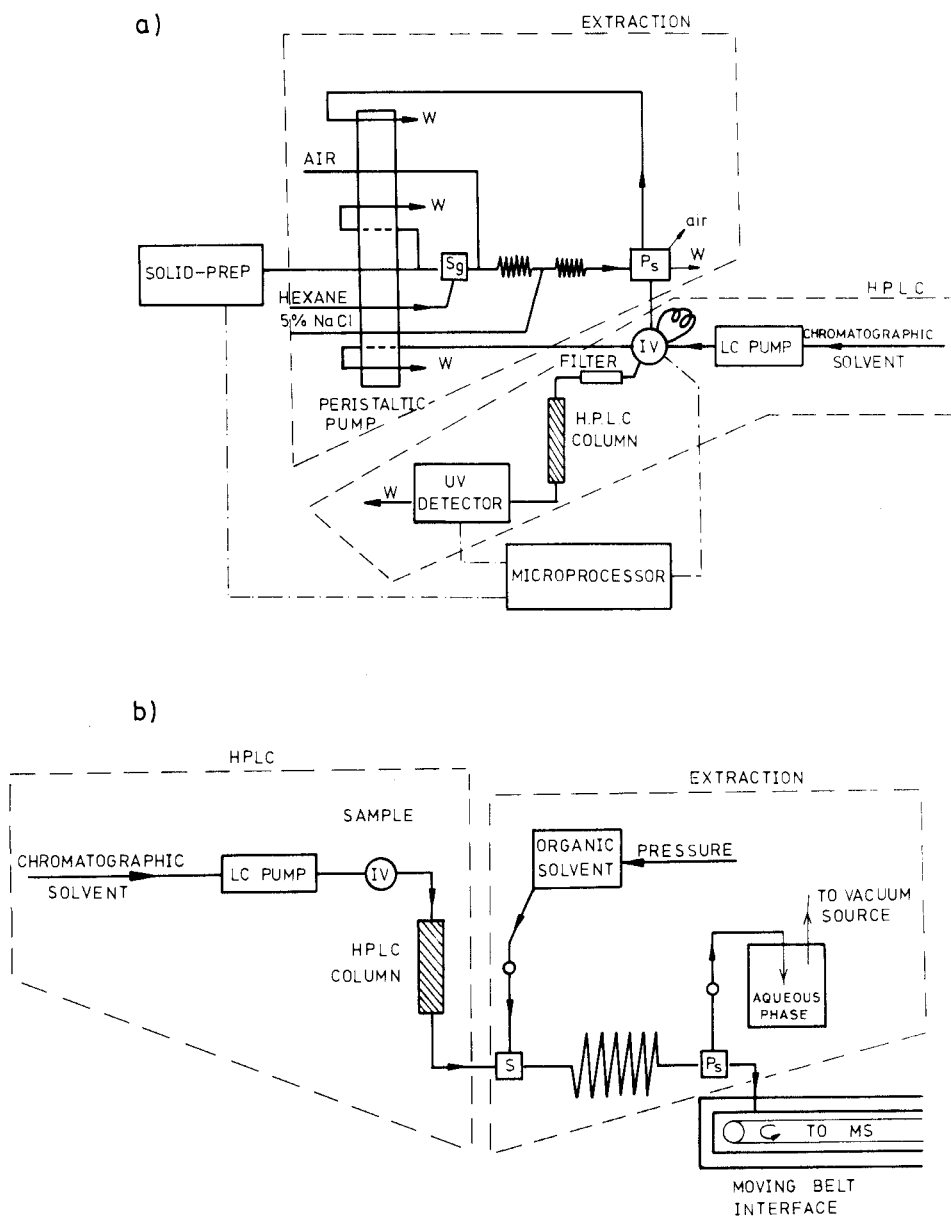
**Fig. 4.27** Incorporation of a continuous liquid-liquid extraction module (a) prior to and (b) after the chromatographic column in HPLC. (A) Determination of fat-soluble vitamins in pharmaceutical preparations; (B) post-column solvent change-over. (Reproduced from [28] with permission of Elsevier).

tions. The other possibility involves using extraction modules for solvent changeover. As a significant fraction of HPLC work is conducted under reversed-phase conditions, the direct coupling of a liquid chromatograph to a mass spectrometer is a problem which remains to be solved. The major limitations of these methods is their inability to effectively remove, prior to entering the mass spectrometer, either the solvent or other non-volatile constituents, or both. Figure 4.27b shows the scheme of an HPLC-MS system using a continuous extraction interface in the absence of air segmentation. A typical moving belt receives the organic phase with the extracted ion pairs.

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# 5

# Automatic continuous analysers. I.

# Air—segmented flow analysers

## 5.1 INTRODUCTION

Continuous automatic analysers (CCA) are characterized by the fact that the transport of samples and reagents along the system is effected by establishing a gas or liquid stream flowing through the straight and coiled tubes making up a typical manifold. As will be seen in this chapter and next two, the sample and reagent(s) can be mixed in a number of ways, and a variety of intermediate operations from the mere halting of the flow to the incorporation of continuous separation units (dialysers, extractors, etc.) can be involved in the operation of this type of analyser which also typically uses continuous detection systems featuring a flow-cell through which the stream carrying the reacting mixture is passed —some techniques such as atomic spectroscopy, though, require no flow-cell. The transient signals provided by the sensing system, whose time-dependence and shape are obviously a function of the operational mode used, are collected by a conventional recorder or a microprocessor. Some of the features of these signals (e.g. the peak height, width and area) are related to the analyte concentration.

Functionally, these analysers resemble liquid and gas chromatographs, although their foundation is markedly different [1,2]. They also differ from discrete automatic analysers (DAA) in various respects, namely the fashion in which samples are transported and mixed with diluents and reagents, the manner in which carry-over between samples and reagents is avoided and the type of detection used —continuous *vs.* discrete. These differences obviously result in others (e.g. design, cost, versatility) that were commented on in preceding chapters and are deal with in greater detail here.

In Table 5.1 is shown the classification of automatic continuous methods based on the way in which carry-over between samples successively introduced into the analyser is avoided. As can be seen, there are two general groups:

*Continuous segmented methods* avoid carry-over by use of air bubbles establishing physical separations (segments) along the continuous flowing stream. These methods are typically implemented on Technicon AutoAnalyzers and Skalar assemblies. Samples are introduced sequentially by aspiration with a moving

articulated pipette. Formerly known by the generic name of 'continuous-flow analysers', they should be referred to more properly as 'segmented-flow analysers', although the term 'segmented' is also used to describe the continuous extraction systems presented in Chapter 4 —these, however, involve liquid-liquid interfaces.

**TABLE 5.1**

Classification of automatic continuous methods

| According to whether or not the streams contain bubbles | Sample introduction | | | |
|---|---|---|---|---|
| | According to procedure | According to time | Nature of flow | Name |
| Segmented | By aspiration | Sequential | Continuous | Segmented flow analysis (SFA) |
| | By injection | Sequential | Continuous | Flow-injection analysis (FIA) |
| | | Sequential | Discontinuous | Stopped-flow kinetic methods |
| Unsegmented | By aspiration | Continuous | Continuous | Completely continuous-flow analysis (CCFA) |
| | | Sequential | Continuous | |
| | | Sequential | Discontinuous | Controlled-dispersion flow analysis (CDFA) |

*Continuous unsegmented methods* are characterized by the absence of air bubbles from the flowing system and by their greater technical simplicity. The way in which carry-over is avoided differs from mode to mode. Thus, in flow-injection analysis [2,3], the samples are introduced sequentially by injection or insertion of a preset volume into an uninterrupted liquid stream of reagent or carrier-diluent. In stopped-flow kinetic methods, the samples are injected simultaneously with the reagent and the mixtures of both are transported at high speed to the measuring cell, where the flow is stopped for as long as detection lasts. The entire analytical operation —including data collection—

thus takes place in a very short interval (of the order of a few hundredths or thousandths of a second) [4]. These methods are usually implemented for carrying out physico-chemical studies, on complex, precise and costly commercialy configurations, although cheaper alternatives providing comparable results have been described [5]. In completely continuous methods, the sample and reagents are aspirated continuously into the flow system and no discontinuities resulting from injection, sample changeover or flow halting are introduced [6]. In some continuous unsegmented methods with sequential continuous aspiration of the sample, this is introduced until the transient signal sought is obtained, after which it is replaced with a fresh one, thereby introducing a discontinuity, distinguishing these from completely continuous methods. There are also continuous unsegmented methods based on the discontinuous aspiration of the sample, whose sequential introduction into the system is accomplished by periodical stopping of the flow rather than by aspiration of air bubbles as in continuous segmented methods. In these so-called 'controlled-dispersion flow methods', intermittent pumping serves the same function as the injection valve does in continuous segmented methods [7].

As stated above, automatic continuous segmented analysers were the earliest to be developed in the field of automatic methods of analysis. They originated from the transcendental contribution of Skeegs in 1957 [8], materialized in the first continuous dynamic measuring system with sequential introduction of samples and the use of a flow-cell. Sample carry-over was prevented by segmentation with air bubbles introduced between successively aspirated samples. Skeeg's original idea was consolidated in the development and massive commercialization of Technicon AutoAnalyzers and later Skalar assemblies and others. For many years, these were the only alternative available for the automation of high-throughput control laboratories. However, the later development of other modes of automatic continuous analysis has demonstrated the invalidity of Skeeg's exclusivistic approach. Thus, alternatives such as FIA or completely continuous methods clearly excel over segmented methods in performance (rapidity, reagent saving, cost, flexibility). All things considered, due credit should be given to Skeeg's contribution, the starting point for the systematic development of laboratory process automation.

Unlike with discrete or batch configurations, the nomenclature used with continuous configurations is not quite correct taking into account the clear distinction between the terms 'analysis' and 'determination', established by Pardue [9] in his hierarchical view of Analytical Chemistry (see Chapter 1). Thus, terms such as 'continuous-flow analysis', 'segmented-flow analysis' or 'flow-injection analysis' are not meant to describe the overall analytical process insofar as they do not include the preliminary sampling and sample treat-

ment operation. Hence, the word 'analysis' should be dropped from these expressions.

## 5.2 GENERAL SCHEME OF AN AIR-SEGMENTED ANALYSER

Automatic air-segmented analysers are characterized by the use of one or several liquid streams (diluents, washing solutions, reagents) where the sequentially aspirated samples are introduced and spaced by means of air bubbles aimed at avoiding the undesirable carry-over [10-12].
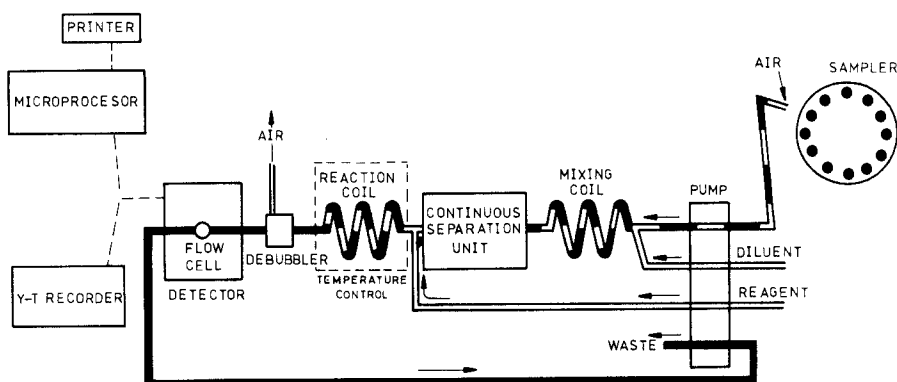


**Fig. 5.1** Scheme of an automatic continuous segmented flow analyser.

This type of analyser is usually modular in nature and consists of a series of elements (apparatus, instruments) coupled on-line to one another. Figure 5.1 shows the essential components of a straightforward Technicon AutoAnalyzer, namely:

*Sampling system*, which consists of a sample turntable and moving articulated aspiration probe, and differs very little from the generic designs described in Chapter 3.

*Propelling unit(s)*, aimed to 'move' the analyser. They are generally peristaltic pumps, although their function can also be served by piston pumps and the pressure exerted by a gas or gravitational force. In short, they are meant to set and keep several streams in motion. The flow-rate of such streams should be regulatable and maintained as constant as possible, which is normally accomplished by using flexible tubes that withstand the mechanical pressure to which they are subjected.

*Reaction-mixing coils, viz.* pieces of polyethylene, PTFE or glass tubing where the mixing of reactants and the analytical reaction take place. Their length and the flow-rate of streams circulated through them determine the time over which the reacting mixture 'resides' in them and hence the sampling frequency, inversely proportional to the time required for the complete analytical reaction.

*Heating system,* usually consisting of thermostated baths or electrical wires wrapping the coils to favour the development of the analytical reaction.

*Continuous separation systems,* optional elements such as dialysers, liquid-liquid extractors, sorption or ion-exchange columns, filters, etc. that can be placed before the reaction coils to remove potentially interfering species.

*Debubbler,* which functions to remove the previously introduced air bubbles in order to avoid parasitic signals produced by their interfaces upon reaching the detector. They are not normally required in the more recent designs as the signals from the detector are usually handled by a computer capable of discriminating between these undesirable signals and those actually corresponding to the reaction mixture.

*Continuous detection system,* usually of optical (colorimetric, photometric, fluorimetric) or electroanalytical (potentiometric, voltammetric) nature. The design of the flow-cell, when required, must be suited to the particular detection system used.

*System for data collection and treatment,* which should be prepared to operate in a continuous fashion and be as simple as a typical Y-T recorder or as sophisticated as an advanced microprocessor carrying out both operations and eventually delivering the results as required.

Although some of these elements, such as the heater, continuous separator, debubbler or microprocessor, are not indispensable, the rest are dependent on the design of continuous segmented analysers.

## 5.3 GENERAL CONCEPTS

The major features of a determination carried out on an automatic segmented-flow analyser, namely precision and rapidity, are highly influenced by technical factors such as the extent of carry-over and mixing of reactants, the time during which the reacting plug remains in the system, etc. Below is a detailed discussion of a series of concepts key to the performance of this type of analyser and hence to the results they provide.

### 5.3.1 Foundation

Measurements carried out with these analysers are made under a dual equi-

librium: physical (homogenization of the sample-reagent zone between two con-
secutive bubbles) and chemical (the analytical reaction has reached equilib-
rium before the reacting plug reaches the detector, except in reaction-rate
methods). Such determinations are thus non-kinetic, unlike those implemented
by application of other continuous methodologies such as FIA. Hence, the de-
sign and functioning of these analysers should be aimed at obtaining  steady-
state signals (i.e. constant over a preset time). Despite their non-kinetic
nature, continuous segmented methods are indeed *dynamic* insofar as the flow
motion is a physico-kinetic aspect to be considered.

### 5.3.2 Analytical signal

The typical transient signal provided by continuous segmented analysers is
shown in Fig. 5.2. It is obtained upon passage of the reacting mixture zone,
flanked by two reagent or washing solution zones —the air bubbles having been
previously removed— through the detector. As can be seen, it consists of
three parts: a rising portion, a plateau (steady-state signal) and a falling
portion merging again with the baseline. In theory, this signal should be a
broken line forming a rectangle with the baseline. The divergence is the
result of various factors, but basically of the mixing between segments after
the air bubbles are removed.
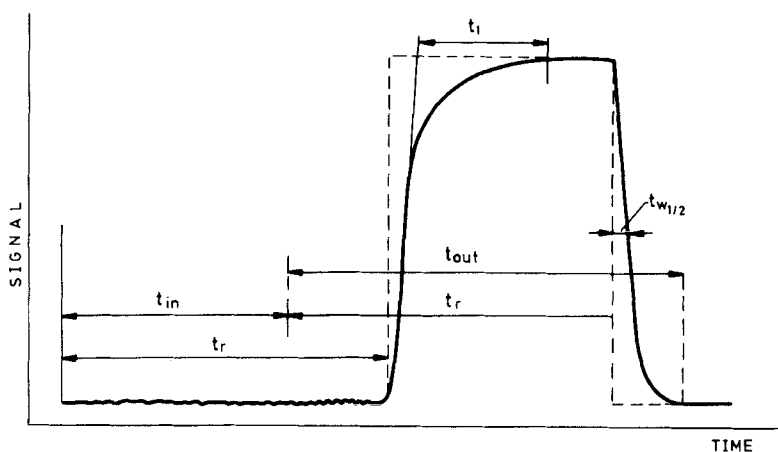


**Fig. 5.2** Characteristic profile of  transient  signal provided by an
automatic continuous  segmented analyser  and parameters  defining it.
The dotted line represents the theoretical response.

Figure 5.2 also presents a series of characteristic parameters: $t_r$, the
time elapsed between the start of sample aspiration and its arrival at the de-

tector; $t_{in}$, the aspiration time over which the withdrawing needle is submerged in the sample vial; and $t_{out}$, the interval during which the aspirating tip remains outside the sample vial withdrawing air and washing solution. There are another two parameters of great significance to the transient signal, namely the lag-phase ($t_L$) and the half-washing time ($t_{W1/2}$). The lag-phase is related to the first portion of the signal and is defined as the interval elapsed between the start of the signal and the obtainment of the steady-state signal, i.e. the deviation from ideal behaviour in this zone. The half-washing time is defined as the time required for the signal at a given point to be halved. After much study, the rising portion of the transient signal has been found to respond to an exponential expression of the form

$$\Delta C/\Delta t = R(C_E - C_t)$$

where $C_E$ is the equilibrium concentration and $C_t$ that corresponding to a given time t. In practice, the analyte concentration is calculated from the steady-state signal, while the signal area, in contrast to other continuous methodologies, is rarely used as it is ostensibly divergent from the theoretical value.

### 5.3.3 Sample carry-over

Carry-over in continuous segmented-flow analysers arises essentially from the undesirable mixing of successive samples in three parts of the system, namely:

(a) The aspiration system, the tip of which is liable to be contaminated —both internally and externally— by previous samples unless one of the washing mechanisms described in Chapter 3 is employed.

(b) The flow system, the most frequent source of mutual contamination between samples. As can be seen in Fig. 5.3a, a static, thin liquid film prevents direct contact of air with the tubing walls, thereby generating a heading sample residue. Upon arrival at the same point, part of the first sample is mixed with and incorporated into the segment of the second.

(c) The connection between the debubbler and the flow-cell. Despite its very short length, it favours mixing through the absence of any physical separation between the samples. The linkage should be as narrow and short as possible in order to avoid axial diffusion, and contain few elbows or void volumes, which favour the mixing by creating turbulence.

Figure 5.3b shows the effect of carry-over on the transient signals, contrasted with the theoretical situation. As can be seen, substantial carry-over results in strongly overlapped, analytically unusable signals. The occurrence of carry-over in a segmented configuration (Fig. 5.4) can be determined by se-

quentially introducing three samples ($S_1$, $S_2$, $S_3$), the first and last of the same low concentration and the intermediate one ($S_2$) of a much higher concentration. In the absence of mutual contamination, the steady-state signals corresponding to the first and third samples should be identical; if the third is higher than the first, the continuous segmented analyser is ostensibly subject to carry-over. The fact that the signal between samples may not reach the baseline is not generally of concern in routine determinations as long as the signal attains equilibrium, i.e. provided it reaches the plateau on which the analytical calculations are based.
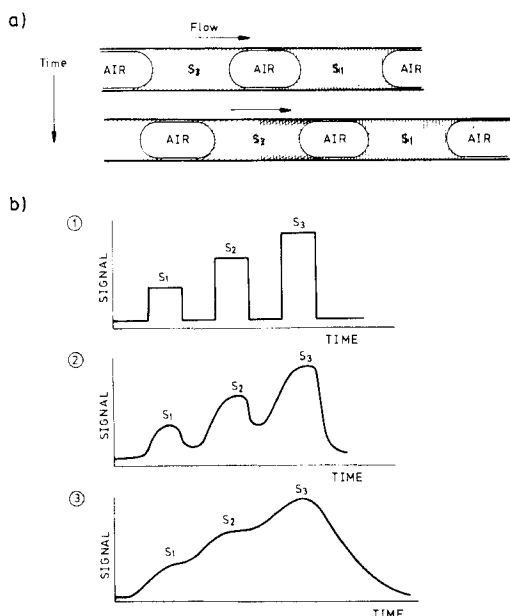


**Fig. 5.3** Carry-over in air-segmented flow configurations. (a) Contamination of sample $S_2$ by $S_1$ in the flow system produced by the thin liquid film between air and the tube walls. (b) Effect of carry-over on the transient signals yielded by three consecutive samples ($S_1$, $S_2$ and $S_3$). The effect increases from $S_1$ (theoretical situation) to $S_3$.

In theory, the steepness of the rising and falling portions determines the extent of overlap between transient signals. The steeper the signals are (theoretical situation), the lower is the probability of overlap. A measure of carry-over is given by the so-called degree of interaction

$$\phi = \frac{t_b - t_L}{t_{W1/2}}$$

a)



b)
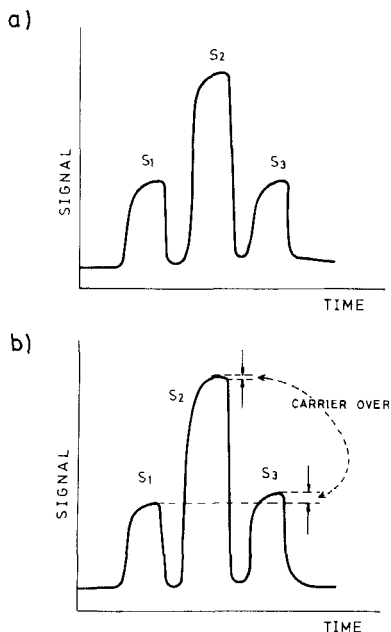


**Fig. 5.4** Experimental checking of the occurrence of carry-over between samples introduced successively into a continuous segmented analyser involving the use of three samples ($S_1$, $S_2$, $S_3$) of concentration $C_{S1}$, $C_{S2}$ and $C_{S3}$ (=$C_{S1}$). (a) Negligible carry-over; (b) significant carry-over (requires correction).

where $t_b$ is the overall time elapsed between the aspiration of two successive samples. The longer is $t_b$ and the shorter are the lag-phase and the half-washing time, the lower the probability of sample interaction and hence carry-over will be. The influence of $t_{W1/2}$ is more marked than that of the other two parameters insofar as it is a measure of the slope of the rising and falling portion of the transient signal. The extent of overlap decreases with increasing degree of interaction according to

%overlap = $2^{-\phi} \times 100$

Air bubbles are therefore not completely efficient in preventing carry-over in these configurations. The proof of this is that Technicon introduced an intermediate washing solution almost since they started the commercialization of its Autoanalyzers. This washing solution is used by the aspirating tip in the following sequence, illustrated in Fig. 5.5 alongside the flow profile

obtained prior to and after the mixing with the diluent and reagent streams: (1) aspiration of air, (2) aspiration of a sample (S1), (3) aspiration of air, (4) aspiration of washing solution, (5) aspiration of air and (6) aspiration of the next sample (S2). This cycle is repeated until the last sample in the sampler has been processed. The intermediate washing operation decreases carry-over in the three zones where it usually appears by (a) washing the aspirating tip internally and externally, (b) allowing segments of the sample to be transferred through the liquid film thanks to the substantial dilution of the small 'delayed' amount of the heading sample and (c) establishing a liquid zone between samples which drastically reduces the possibility of reaction zones interacting after the debubbler.
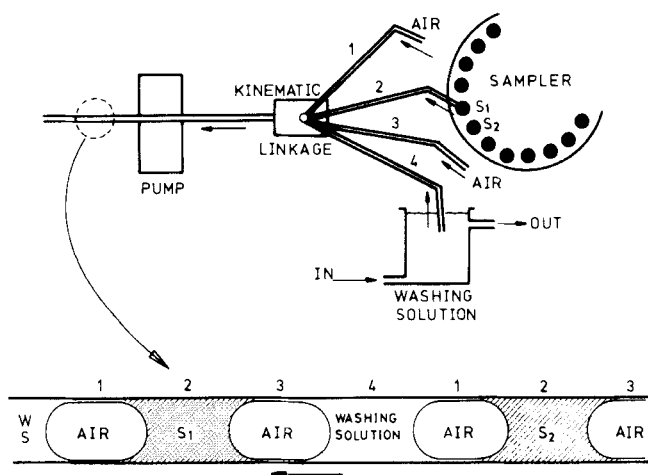


**Fig. 5.5** (Top) Use of an intermediate solution to avoid carry-over between samples. (Bottom) Flow profile after aspiration of two samples S1 and S2.

The flow-rate (mL/min) of the reacting mixture on passage through the flow-cell, determined by the flow of the waste stream through the pump, is another major factor influencing carry-over. Figure 5.6 illustrates the effect of low flow-rates on the transient signal profile. In addition to shortening the time required for attainment of the steady-state signal, they lengthen the time of restoration of the baseline, thereby increasing the probability of overlap with the signal yielded by the next sample. Insofar as the identity of the segments is lost after the debubbler, low flow-rates give rise to markedly increased axial diffusion, thus extending the zone of occurrence of each sample and increasing the risk of mutual contamination. Higher flow-rates result in more efficient sweeping and hence in narrower transient signals. The deviations in the rising portion of the curve are due to the mixing with the preced-

ing sample segment or the dilution with the carrier or washing solution, as in Fig. 5.6, where only one sample was used.
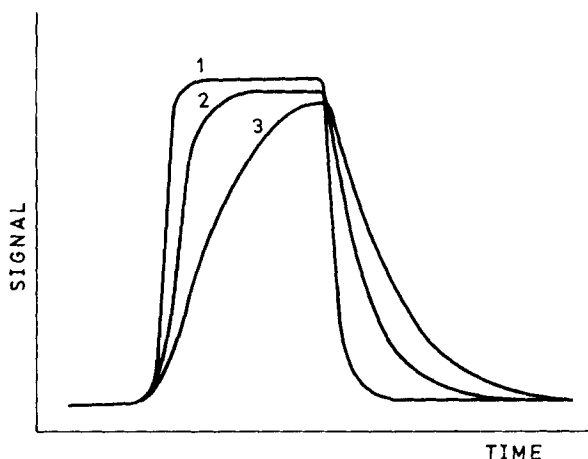


**Fig. 5.6** Influence of the flow-rate on sample carry-over in a continuous segmented configuration. Carry-over decreases from 1 to 3.

### 5.3.4 Sampling frequency

The sampling frequency, commonly expressed as the number of samples processed per hour, is one of the features whereby the performance of an analyser is evaluated. According to the statements above, a determination will be feasible only if the transient signal reaches the steady state in a time sufficiently short to permit its recording or electronic acquisition. This, in turn, involves minimizing $t_{in}$, the sample aspiration time, and $t_r$, *viz.* the interval over which the sample resides in the system —this, however, should not be so short as to prevent physical and chemical equilibrium to be reached. Carry-over is yet another factor limiting the sampling frequency: on the one hand, the use of an intermediate washing solution considerably delays the sampling operation; on the other, long lag phases and the half-washing times will impose an increase in the sample volume to be aspirated, thereby reducing the introduction frequency.

A detailed study of all the factors that influence the sampling frequency complemented with a computerized procedure for optimization of continuous segmented configurations was reported by Angelova and Holy [13].

### 5.4 FACTORS AFFECTING THE SIGNAL QUALITY

Although the influence of the different experimental variables on the oper-

ation of a continuous segmented analyser was commented on above, it is worth considering the repercussions of such variables on three aspects of decisive importance to the quality of the signal and hence to the analyser performance. These three aspects are the sample dispersion, sample-reagent mixing and flow stability.

### 5.4.1 Sample dispersion

The term 'dispersion' is used to refer to the spread of an aspirated sample in a flow system, chiefly as a result of the stationary liquid film detracting from the separating efficiency of air bubbles. Snyder and Adler [14-16] studied the influence of a series of experimental variables on the dispersion. Such variables were classified as (a) analyser variables (tube diameter, $d$; flow-rate, $q$; residence time, $t_r$; segmentation rate, $n$, expressed as the number of bubbles circulating per second) and (b) sample variables (viscosity, $\eta$; surface tension, $\gamma$; molecular or ionic diffusion coefficient, $D$). Of these variables, the flow-rate, tube diameter, segmentation rate, viscosity and residence time influence carry-over to the greatest extent. The dispersion is directly proportional to $t_r$, $d$ and $n$, which should therefore as small as possible to ensure minimum dispersion. The flow-rate also has a great influence on the dispersion; as it is inversely proportional to $t_r$ and hence to the dispersion, the higher it is the less the carry-over will be. In general, any alteration in the above-mentioned variables is to the detriment of another of the major features of an analyser, namely the sampling frequency.

In the above-mentioned studies, Snyder and Adler used a theoretical model which they contrasted with experimental results. They evaluated the dispersion by assuming the transient signal to be Gaussian (see Fig. 5.3b). By expressing the bandwith as a standard deviation ($8\sigma$ and $4\sigma$ for signals with and without a plateau, respectively), the dispersion can be related to the analyser and sample variables through

$$\sigma = \left[ \frac{538 d^{2/3}(q+0.92d^3\eta)^{5/3}\eta^{7/3}}{\gamma^{2/3}qD} + \frac{1}{n} \right] \left[ \frac{2.35\ (q+0.92d^3\eta)^{5/3}\eta^{2/3}t_r}{\gamma^{2/3}qd^{4/3}} \right]$$

where $d$ is in cm, $q$ in mL/s, $n$ in bubbles/s, $\eta$ in Poise, $\gamma$ in dyne/cm, $t_r$ in s, $D$ in cm/s and $\sigma$ in s. Fig. 5.6 shows the variation of the standard deviation as a function of the number of bubbles per centimetre (logarithmic scale) at different flow-rates and constant $t_r$=500 s and $d$=1 mm. As can be seen, there is a minimum (optimum) standard deviation for each flow-rate. Variables should be optimized to decrease bandwidth and hence $\sigma$ as far as possible.

The maximum sampling rate (number of samples processed per hour) achievable with no signal overlap is given by

$v = 3600/4\sigma = 900/\sigma$      (signals with plateau)
$v = 3600/8\sigma = 450/\sigma$      (signals without plateau)

Obviously, these sampling rates can be safely augmented (by 20-30%) as a slight overlap between the ends of two signals is acceptable provided that it does not affect their steady-state portions.

### 5.4.2 Sample–reagent mixing

The basic principle behind automatic continuous segmented analysers is the homogenization of the sample-reagent-diluent zone between two bubbles. This physical equilibrium is reached thanks to the existence of a minimum residence time, $t_r$, over which two physical phenomena contribute to the homogenization of the continuously introduced sample. On the one hand, the compressibility of air bubbles gives rise to a turbulent flow regime which fosters mixing. On the other, tubes are helically coiled to favour radial diffusion through the centrifugal force additional to the sweeping effect of the flowing stream, which clearly shortens the homogenization time. The factors that most strongly influence efficient mixing in these continuous systems are the tube diameter, coil diameter, segment length, flow-rate (or tube length) and the characteristics of the flowing solution (viscosity, density, diffusion coefficients of the reactants).

### 5.4.3 Flow stability

A regular, stable flow profile is indispensable for the obtainment of reliable and reproducible results from a continuous segmented analyser. In other words, all circulating liquid segments should be of the same length, thereby ensuring the constancy of the flow-rate and the bubbling rate. Irregularities in the length of the segments are chiefly caused by oscillations in the volume at which the sample is mixed with the reagent and the diluent, which in turn arise from: (a) flow-rate inconstancy; (b) pulsations of peristaltic pumps; (c) temperature variations, which result in changes in the compressibility of bubbles; (d) sample scarcity (too short aspirations); (e) dirt in the sample or reagent tubes, which may clog the system; and (f) the use of liquids that do not 'wet' the tube walls. The alterations resulting in deviations from ideal flow profiles are chiefly caused by peristaltic pumps (a, b). Keeping the flow-rate constant is no easy task as it requires frequent checking and replacement of the pump tubes as frequently as recommended by the manufacturer. The supression of pulses is cumbersome: it usually requires the use of

special devices fitted to the pump outlets, although their disturbing effect can also be avoided by synchronizing them with the aspiration of air into the system.

The efficient mixing of reactants is no doubt one of the chief limitations of continuous segmented analysers, which require more frequent checking than their unsegmented counterparts and have much longer warm-up times (between 0.5 and 1 h), which delay the start of determinations.

## 5.5 ESSENTIAL COMPONENTS OF AN AIR-SEGMENTED ANALYSER

Although the automatic continuous segmented analysers marketed by Technicon are by far the best known, other firms manufacture a variety of configurations worthy of note (e.g. the FlowComp 1500 from Carlo Erba Instrumentazione). There is also a recent trend to develop continuous analysers capable of implementing segmented and unsegmented methodologies as required. Such is the case with the more recent assemblies developed by Skalar, a firm that formerly concentrated on segmented configurations but which now manufactures a range of automatic hybrid SFA-FIA systems adaptable to a larger variety of analytical applications. On the other hand, the later novelties introduced by Technicon in this area involve mere technical modifications of earlier segmented configurations.

All these configurations consist of a series of common basic components already briefly commented on in Section 5.2 and described in greater detail here.

### 5.5.1 Sampling system

As stated above, the sampling operation is carried out with the aid of a moving aspirating tip in continuous segmented systems. However, unlike in other configurations, some air is also withdrawn between sample aspirations. The volume taken by the tip can be quantized in two ways, namely:

(a) Over a fixed time, i.e. by keeping the aspirating tip submerged in the solution (sample, washing solution) for a preset interval, so that if the flowrate is constant, the volume taken in each operation will be exactly the same. Alhough it is the commoner option, its efficiency depends critically on the smooth functioning of the propelling unit.

(b) By taking a fixed volume every time by use of two pairs of conductimetric electrodes strategically placed close to the aspiration system. The passage of a bubble between them gives rise to an electrical signal which switches a simple electronic assembly governing the tip motion and the sample tray turn. This alternative, although technically more complicated, yields more reproducible results and is less subject to the functioning of the propelling unit.

Most samplers are electronically or computer-controlled, so that they allow programming of the aspiration probe and the turntable. Thus, the Technicon Sampler II permits the selection of the sample-to-washing solution volume ratio, which can be varied between 1:6 and 6:1, and the sampling rate (20, 40 or 60 samples/h). Later models such as the Sampler IV are even more flexible and work over wider ranges of the above-mentioned parameters. The SOLIPpepII module (also manufactured by Technicon), described in Chapter 3, allows direct sampling of solid samples in automatic continuous analysers.

### 5.5.2 Propelling system

The peristaltic pump is the device most frequently used to propel fluids along continuous segmented systems. Their functioning is based on the squeezing of flexible plastic tubing by means of a series of rollers, which starts the flow of the enclosed liquids as a result. They normally permit working with several streams (4-29 lines). The flow-rate of the circulating liquids is determined by the internal diameter of the tubes; as a rule, it is controlled by selecting the appropriate tube diameter, although some pump models are suitable for this purpose thanks to their selectable rotation speed. The pump tubes are one of the elements that most decisively influence the performance of a continuous analyser. On the one hand, their continuous wear through mechanical friction results in the progressive alteration of their internal diameter and hence of the flow-rate of the liquids circulating through them, which compels the user to replace them fairly frequently. On the other hand, there is the risk of the circulating fluids interacting with the tube walls, the constituent material of which must be resistant to potentially aggressive agents (organic solvents, oxidizing acids, etc.).

There are other alternatives to the peristaltic pump for establishing the flow in continuous analysers, a detailed description of which is given in the authors' book on FIA [2].

### 5.5.3 Reaction system

This function is served by compact, helically coiled tubes of a given diameter for convenient use. They are readily connected to the other elements of the system, so that void volumes are minimized. Occasionally, they are heated by means of electrical wires or a thermostated bath.

### 5.5.4 Separation unit

One of the most appreciated advantages of continuous analysers is the possibility of incorporating efficient continuous separation units, which considerably broaden their scope of application in improving two key analytical as-

pects, namely selectivity and sensitivity —through preconcentration. Over 70%
of all the clinical applications of continuous segmented analysers reported to
date used a continuous dialyser (see Chapter 4 for a detailed description).
This requires the segmentation of the two streams flowing through it: the car-
rier (sample) and the acceptor (generally containing the reagent and eventual-
ly driving the reacting mixture to the detector. Other separation units such
as liquid–liquid extractors are less commonly used with continuous analysers.
However, Technicon and Skalar manufacture the elements required to implement
continuous extractions (segmenter, extraction coil, phase separator, etc.)
[10]. Even less frequent is the use of distillation [17–19] and filtration
[20,21] units. Figure 5.8 depicts the distillation system marketed by Skalar.

a)                                                              b)
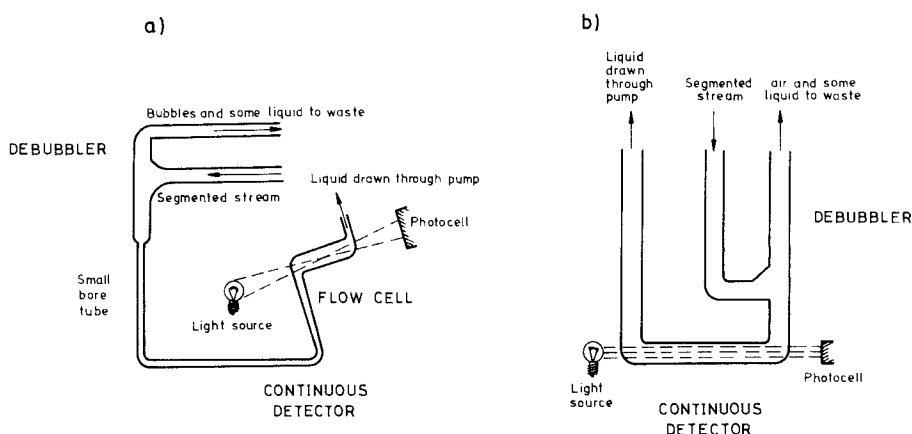


**Fig. 5.7**  Debubbling  and  detection  (colorimetric)  systems  used  in  (a)
Technicon AutoAnalyzers and (b) Skalar assemblies. (Courtesy of Techni-
con and Skalar).

### 5.5.5 Debubbler

The air introduced in segmented systems is normally removed with the aid
of a high–precision device generally built into the flow-cell (Fig. 5.7). Its
functioning is rather simple: it splits the flowing stream into two channels,
one pointing upwards and allowing the removal of the bubbles and some liquid,

and another carrying the gas-free liquid flow. Needless to say, the smooth operation of this unit requires the incoming and outgoing flow-rates to be precisely regulated. Hence, the flow emerging from the detector in continuous segmented analysers may not be sent directly to waste, but must rather be previously passed through a tube of the peristaltic pump regulating the outgoing flow-rate. Figure 5.7a depicts the channel narrowing after the debubbler and before the flow-cell, aimed at preventing the dispersion of the sample zones sequentially reaching it and hence avoiding carry-over. Later Technicon models use no debubblers, as their computerized data acquisition allows the convenient discrimination of the air bubbles passing through the detector.
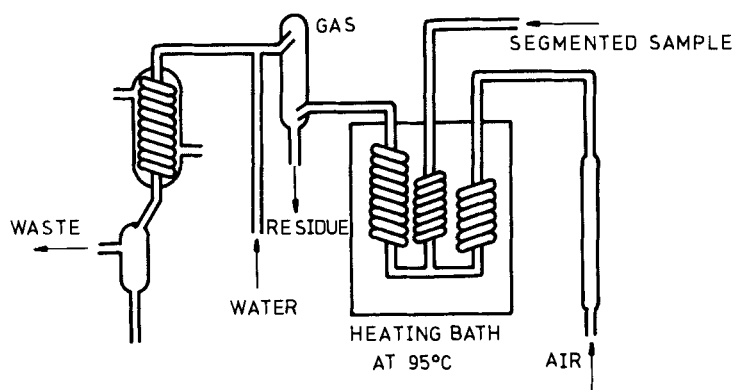


**Fig. 5.8** Continuous microdistillation assembly for incorporation into continuous segmented configurations. (Courtesy of Skalar).

### 5.5.6 Detection system

Continuous segmented analysers are used with a variety of detection systems which generally consist of a flow-cell accommodated in optical or electroanalytical instruments, whether all-purpose or specific —the latter are the precursors of HPLC detectors. Technicon AutoAnalyzers use the detection system depicted in Fig. 5.7a for photometric or colorimetric sensing —the commoner; on the other hand, Skalar employ U-shaped designs in their assemblies. The use of two photodetectors in the same optical assembly allows the continuous differential monitoring of the analytical signal with respect to the baseline. The fluorescence cells used with these systems are slightly different in design owing to the required perpendicularity between the excitation and emission light paths. Atomic spectroscopic techniques, also implemented on these assemblies, require no flow-cell, the role of which is replaced by a flame,

plasma, etc. Electrochemical detection does require the use of flow-cells placed in such a way that the sensitive part of the working electrode (cylindrical, flat or elongated) is exposed to the flowing stream while the remainder of the electrodes are accommodated in a single small chamber or placed elsewhere in the system, always in contact with the same flowing stream. All these sensing systems are described in greater detail in dealing with the applications of continuous segmented analysers.

Detection in reaction-rate methodologies implemented with these analysers is more complex than with their batch counterparts, where it suffices to allow the reacting mixture to stand in the detector for a given time during which the signal yielded as a result was recorded. Continuous systems can be used in two manners in this respect:

(a) By stopping the flow while the reacting mixture is in the flow-cell. This option is not suitable for continuous segmented analysers as bringing the system to standstill results in significant irregularities.

(b) By use of two detection points placed serially along the system and separated by a delay coil. These give two sequential signals whose separation is a function of the flow-rate and the coil length. This alternative is less precise than the previous one as it does not provide the entire kinetic curve, but only two signal-time pairs, generally insufficient for the accurate determination of the analyte concentration. Figure 5.9 shows the scheme of a continuous detector intended for kinetic measurements with Technicon AutoAnalyzers.
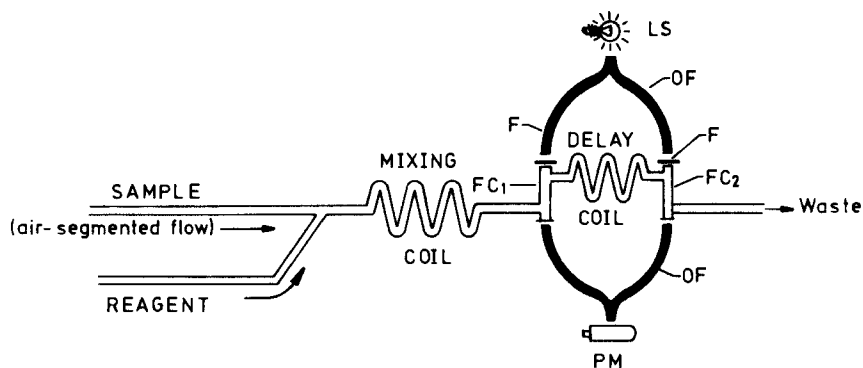


**Fig. 5.9** Assembly for kinetic determinations with continuous segmented analysers without debubbling. LS, light source; PM, photodetector; OF, optical fibre; F, optical filter; FC, flow-cells.

The transient signals provided by the detectors were formerly registered with a strip-chart recorder, which required human participation in the final stage of the analytical process: the operator had to measure signals, contrast samples with standards and match samples and results. The later use of micro-computers for data acquisition and treatment allows the easy delivery of results, expressed in the preselected units, through a printer. Technicon market hardware and software suited to their simpler AutoAnalyzers, which can also be adapted for this purpose with the interesting innovations reported recently [22-25]. Multi-channel models (e.g. SMAC) feature a built-in central computer which, in addition to serving this function, controls the analyser operation.

## 5.6 MULTI-CHANNEL CONFIGURATIONS

The determination of several analytes in the same sample is commonplace in some areas, particularly in clinical chemistry. Multi-parameter designs are thus of great interest to the automation of laboratory procedures, to which commercially available analysers, with the exception of some rather specific examples, are perfectly suited. In continuous segmented analysers, the term
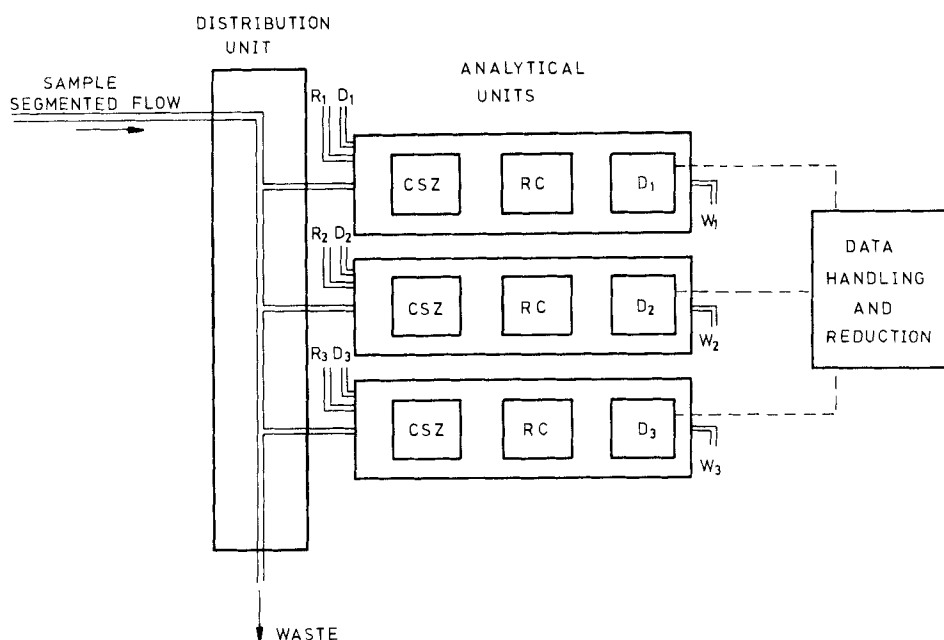


**Fig. 5.10** Foundation of multi-analyte determinations on the same sample in segmented-flow analysers. CSZ, continuous separation zone (optional); RC, reaction coil; D, detectors.

'multi-parameter' can be safely replaced with 'multi-channel' as their operational principle is the use of one channel per analyte to be determined. 'Multi-detection' and 'multi-determination', despite their indiscriminate use by some authors, are not synonyms. In fact, the latter is much wider a term than the former [26].

Automatic multi-channel continuous segmented analysers are based on the following basic principles (Fig. 5.10):

(1) The  aspirated sample volume is split into several channels so that an accurately divided aliquot of sample is driven regularly and evenly together with the other ingredients (air, washing solution) through each channel.

(2) Each channel, led to an independent  analytical unit continuously receiving the ingredients (diluent, reagent) required for development of the analytical reaction, may be fitted with optional separation devices, mixing and reaction coils, heaters, etc., and has its own continuous detection system for sensing the signal yielded by the analyte determined in it.

(3) As a rule, all the channels share a common system for data acquisition and treatment which must therefore be capable of discriminating between signals to assign them correctly to their corresponding analytes. Such discrimination can be effected in two ways, namely:

– By sequential arrival of the signals generated by the different detectors. This is based on a simple principle related to the dynamics of the flow system: the flow-rates and tube lengths are chosen so that the residence time of each aliquot in its channel delays the reception of the signal at the detector sufficiently to avoid coinciding with any of the signals generated by the other channels. This is the operational principle of the Technicon AutoAnalyzer II, capable of determining up to three analytes per sample, and of the SMA 12/60 (Sequential Multiple Analyzer), also made by Technicon and featuring sixteen channels (including four blanks), which can process up to 60 samples per hour [27]. The synchronization of the twelve signals generated by this last analyser requires the highly precise, time-consuming optimization of the features of the analytical units involved —this is facilitated by the use of an oscilloscope.

– By virtually simultaneous arrival of all the signals at a powerful data-acquisition system discriminating between them in a continuous fashion —a microprocessor.

The second option is typically represented by the Technicon SMAC (Sequential Multiple Analyzer plus Computer) I and II, which are expensive and complex analytical systems capable of determining up to 20 parameters per sample at a rate of 150 samples per hour [27]. These analysers consist of the following components, all of which are governed by a central computer:

(1) A sampling unit holding up to 152 vials in a square configuration of several rows much more compact than the typical turntable. An aspirating probe is used to take 450-µL aliquots which allow the determination of up to twenty parameters each. Carry-over is avoided by means of an external cleaning system. Samples are inserted in triplicate and separated by four air bubbles and two zones of washing solution. Hence, the final result is three signals per analyte per sample.

(2) A central dispensing system, namely a tube whose vertical lower part is submerged in the sample-washing solution stream. Along its length (roughly 1 m) are alternately and evenly distributed up to twenty sub-channels.

(3) Twenty  analytical units for individual determination of as many analytes. In addition to independent reagent and diluent streams, heaters, dialysers, etc., they are provided with their own detector, usually photometric (for both end-point and kinetic determinations), and occasionally potentiometric (ISE). In colorimetric determinations each analytical unit posseses one or several flow-cells and a suitable filter.

(4) A single, optical fibre-based system for the generation and detection of the light path. The only light source used is thus shared by all the photometric units. The transmitted light is sent to a single photomultiplier tube and a rotating disc sequentially scans the light beams from each unit, thus achieving the required discrimination. Measurements are carried out at a rate of four  per second. The signals provided by each fibre (three per sample) are stored by the computer.

(5) A microprocessor which controls the functioning of the analyser and is the key part in it. In addition to fast signal acquisition and discrimination it delivers results (e.g. as patient profiles), carries out periodical check-ups, etc.

Because of its high cost and labour demands —it requires the almost continuous attention of one or two operators— the SMAC is only affordable by large hospitals requiring high throughputs.

## 5.7 APPLICATIONS OF CONTINUOUS SEGMENTED ANALYSERS

Continuous air-segmented flow analysers have lost the excellent place they had in the automation of sample processing, particularly in the field of clinical chemistry. The advantages of their batch counterparts have consigned them to relative oblivion. Despite Snyder's predictions in 1980 [28], the advent of cheaper, faster, technically simpler  and more versatile alternatives to continuous analysers (e.g. FIA) was a severe blow to the earlier analysers. However, it is worth noting the remarkable degree of technical perfection reached

by air-segmented designs, which make them very much more reliable than one would expect from their technical complexity.

The potential of these analysers is clearly revealed in Furman's book on the topic [10], the literature on which frequently refers to technical reports published by the manufacturers themselves.

Clinical chemistry is the most extensive area of application of continuous segmented analysers. Indeed, all AutoAnalyzers and some more complex configurations (e.g. SMA, SMAC) were designed for the determination of various parameters in biological fluids from humans with the aim of obtaining a more or less complete analytical profile of each patient. However, these analysers can be readily adapted to other needs in areas such as pharmacological, toxicological, agricultural, food or industrial chemistry by simply replacing some of its elements. Firms such as Skalar make analysers aimed at non-clinical applications, probably so as not to concentrate on field where the competition is already strong. These analysers can also be adapted for use with solid samples by means of the SOLIDprep module II described in Chapter 3 and some other add-ons.

The applications of automatic continuous segmented analysers can also be classified according to the type of detection system involved. Thus, 70–75% of all the methodologies described on this topic used molecular UV absorption spectroscopy (spectrophotometry, photometry), followed by ISE potentiometry (10–15%) and, much less often, nephelometry, fluorimetry, etc. The applications described below were mostly developed with the aid of Technicon technology and are classified according to this criterion —other applications to specific problems related to laboratory processes are described in the corresponding chapters.

The first three determinations described here use the detection technique most frequently employed with continuous segmented systems, namely spectrophotometry, and feature interesting differences in the configurations involved.

Figure 5.11 depicts an assembly used for the determination of ammonia in sea and tap water. It employs no dialyser and is a palpable demonstration of how readily several reagents can be sequentially incorporated into the system with different analytical purposes. The sample, which can be optionally filtered if it contains any suspended matter to avoid introducing disturbances in the system operation, is mixed with EDTA (metal ion masker) and then with phenolate and hypochlorite streams to form the dye indophenol blue, whose colour is finally intensified with a nitroprusside stream. The sample is heated at 50°C prior to passage through the spectrophotometric flow-cell. The determination range is 0.02–2 mg/mL and the sampling frequency achievable is 60 h$^{-1}$ [29].
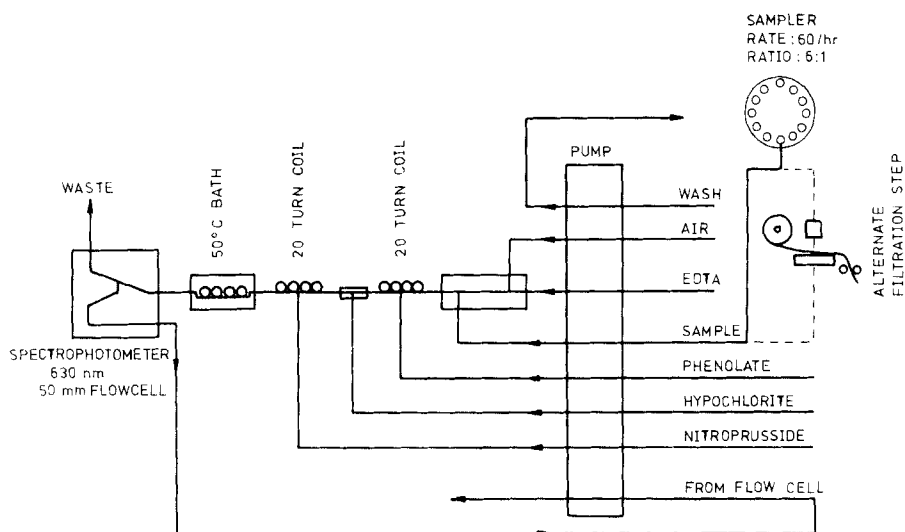
**Fig. 5.11** Continuous segmented manifold for determination of ammonia in various types of water. (Reproduced from [29] with permission of the American Public Health Association).

An alternative determination of ammonia using a continuous dialyser was designed by van Ginkel and Sinnreve [30] for the determination of total nitrogen in plants (see Fig. 5.12). Once dry, the samples are treated with hot hydrogen peroxide until completely dissolved, cooled and placed on the sampler. The aspirated samples are mixed with a sodium hydroxide stream to yield ammonia in the first mixing coil prior to the continuous dialyser, whose membrane allows passage of the gas. This is collected in a water stream, also segmented by air bubbles, which is mixed with a stream of Nessler's reagent containing some tartrate to avoid precipitation of the reaction product. After de-aeration, the absorbance is measured at 410 nm. In this manner, nitrogen can be determined in the range 0–350 mg/L at a rate of 80 samples/h.

The manifold depicted in Fig. 5.13 is used for the determination of chloride ion in sea and tap water [31]. It uses a dialyser to remove interferents. The reagent is a mixture of mercury(II) thiocyanate and iron(III) nitrate, which, in the presence of the analyte, loses the red coloration of the Fe(III)–SCN$^-$ complex as a result of the formation of the stabler Hg(II)–Cl$^-$

complex. The displacement reaction is sensitive enough to allow the determina-
tion of chloride at fairly high concentrations (1-20 g/L).
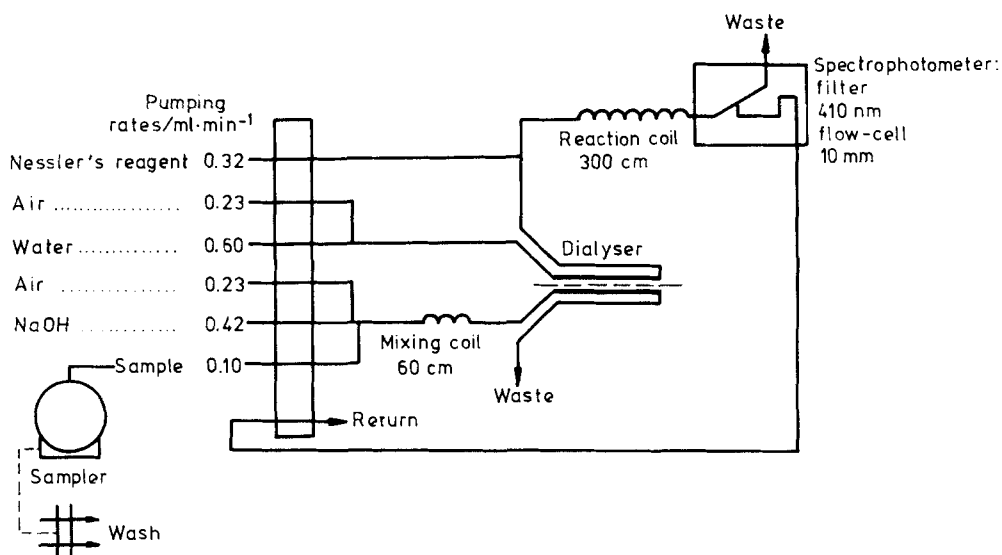


**Fig. 5.12** Continuous segmented flow assembly with continuous dialyser
for the automatic determination of total nitrogen in plants after di-
gestion. (Reproduced from [30] with permission of the Royal Society of
Chemistry).

Fluorimetric detection has scarcely been used with automatic continuous
segmented flow analysers. Figure 5.14 shows the scheme of the configuration
designed by Gfeller and Frey for the automatic determination of amines of phar-
maceutical interest at low concentrations [32]. The determination is based on
the formation of an ion pair with a highly fluorescent anion (9,10-dimeth-
oxyanthracene), continuous extraction into an organic phase (1,2-dichloroeth-
ane) and continuous monitoring of its fluorescence. Between 2 µg/mL and 10
ng/mL can be determined at a sampling frequency of 15-20 h$^{-1}$. The functioning
of this system is identical with that of the continuous unsegmented systems
described in Chapter 3, with the following exceptions: (a) once the extraction
has taken place in EC (Fig. 5.14), the samples are continuously separated in a
T-shaped separator in which both air and the discarded aqueous phase, w$_a$, are

also removed; (b) the organic phase of interest is repumped to the system, which increases the reproducibility of the functioning of the separator PS; (c) an ethanol stream is used to smooth the baseline. The procedure has the advantage that none of the excipients commonly found in pharmaceutical preparations interferes with any of the large variety of amines that can be determined. On the other hand, its performance is strongly bound to the pH.
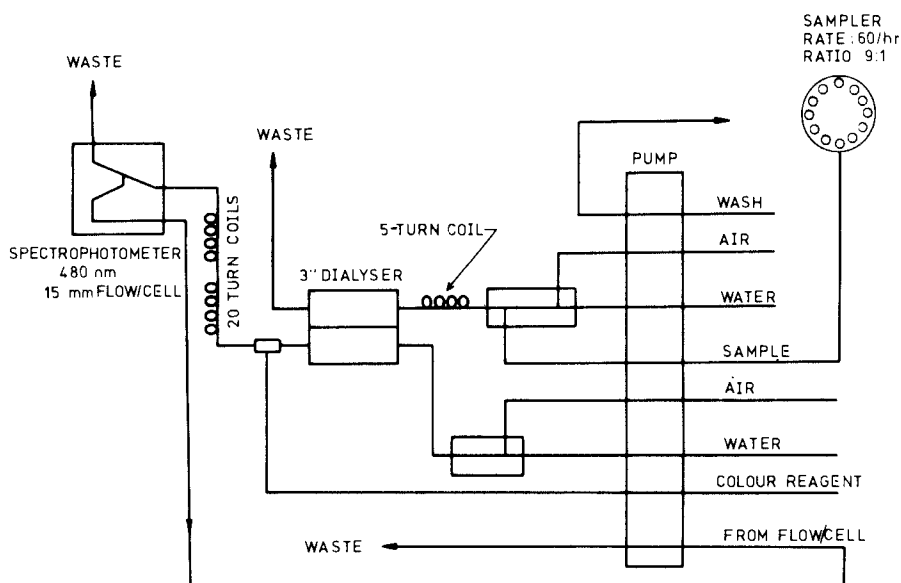


**Fig. 5.13** Continuous segmented configuration for the determination of chloride in water. (Courtesy of Technicon).

Potentiometry with ISEs is the electroanalytical technique most frequently used with continuous segmented configurations. Figure 5.15 shows an assembly designed for the simultaneous determination of sodium and potassium in animal urine [33]. The system is in fact composed of two distinct units for the determination of each analyte. The sample is aspirated and split prior to the peristaltic pump into two channels, which are subsequently mixed with an appropriate buffer (Tris/acetic acid of pH 8.15 for sodium and diethylamine/acetic acid for potassium) and de-aerated before they reached their respective selective electrodes. The reference electrode is an ordinary one and is connected to both streams, into which are introduced two platinum wires connected to the

potentiometer ground to lessen the background noise arising from the flow pulsations generated by the peristaltic pump.
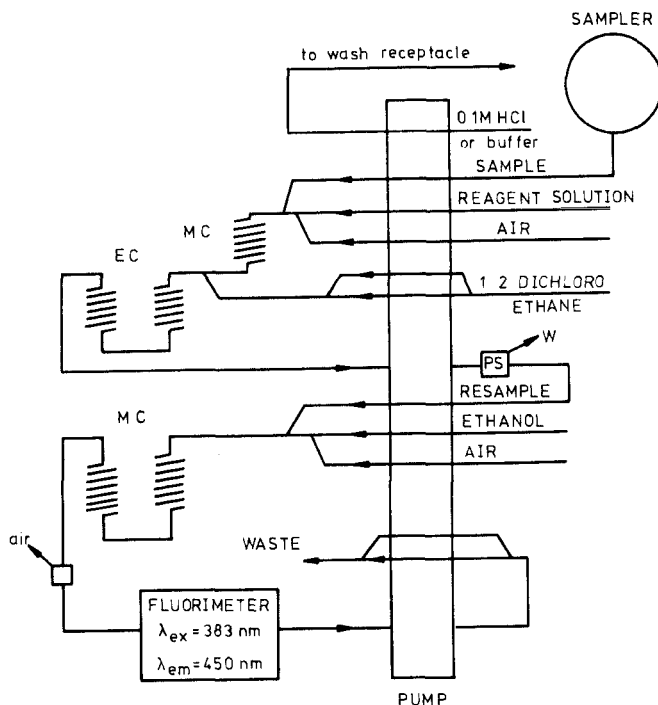


**Fig. 5.14** Continuous segmented assembly with liquid-liquid extractor for the determination of various amines by ion-pair formation. AC, mixing coils; EC, extraction coils; PS, phase separator. (Reproduced from [32] with permission of Springer Verlag).

A potentiometric determination of reducing sugars and glucose in natural and artificial products was reported by Diamandis and Hadjiioannou [34]. The configuration employed is depicted in Fig. 5.16. The sample is aspirated into the system and mixed with a segmented stream of periodate previously merged with a buffer stream. Periodate reacts selectively with $\alpha$-diols under mild conditions by cleaving C-C bonds in the Malaprade reaction. This takes place in a delay coil at a controlled temperature (20°C) for 20 min. After the debubbler, a flow-through iodate-selective membrane electrode continuously monitors the reagent concentration in the flow. The decrease in the signal is proportional to the concentration of the analyte, which can be determined in the range 3-18 mM.
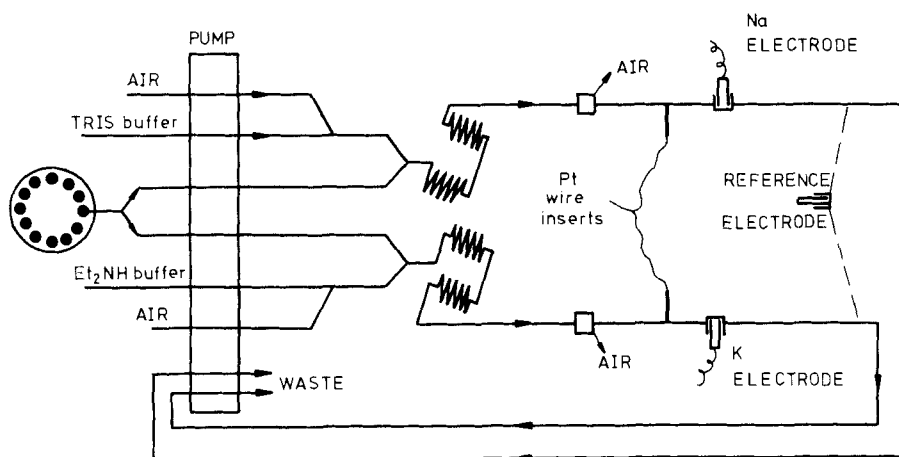
**Fig. 5.15** Continuous segmented-flow assembly for dual potentiometric (ISE) determination. (Reproduced from [33] with permission of the New York Academy of Sciences).

The use of continuous segmented analysers for implementation of atomic spectroscopic techniques offers major advantages over the direct manual aspiration of the sample into the nebulizer, namely: (a) higher sampling rate; (b) lower sample consumption; (c) automatic dilution and addition of ionic supressants and (c) drastic reduction of undesirable effects such as those arising from high salt contents. Figure 5.17 depicts the scheme of a continuous segmented analyser for atomic spectroscopic determinations. It is interesting to note the absence of a flow-cell and the waste, typical of atomic spectroscopic techniques. The sample is diluted with water and the flow is segmented with air bubbles and then mixed mith a lanthanum stream, subsequently being deaerated after a mixing coil and connected to the nebulizer of the atomic absorption spectrometer.

## 5.8 RECENT ADVANCES IN CONTINUOUS SEGMENTED ANALYSERS

To counteract the growing competition to their AutoAnalyzers since the
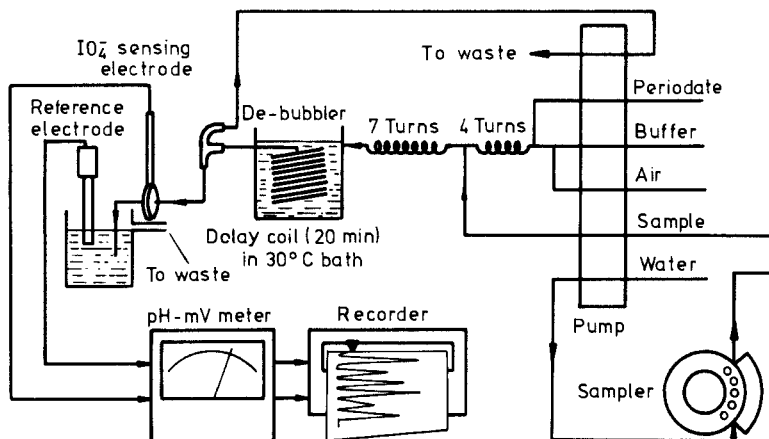
**Fig. 5.16** Continuous segmented manifold using an ISE for determination of reducing sugars and glucose in natural and artificial products. (Reproduced from [34] with permission of the Royal Society of Chemistry).

1970s, Technicon have developed and marketed two alternatives that considerably improve the performance of their earlier models in various aspects. Their functioning is still based on air-segmentation; however, they feature interesting technological innovations resulting from the vast experience of this firm in the field of segmented-flow and batch analysers (RA 1000).

The so-called 'third generation' of Technicon air-segmented flow analysers is represented by the TrAAcs-800, a modular model capable of handling between one and four channels (parameters). The advantages offered by these analysers can be summarized as follows:

(a) Greater automatic control of the different modules by means of a personal computer (an IBM PC, XT, AT or PS) which, with the aid of suitable software, allows total system control, data collection, report generation and system diagnostics.

(b) A sampler with 120 positions based on a combination of sample block and probe movements. Its two-probe capability allows the sample system to aspirate two different samples for simultaneous determinations. A low dead volume overflow washing solution station is provided for high rates of determination with less carry-over.

(c) The use of one peristaltic pump per channel. These pumps feature interesting modifications such as a large number or rollers (60) and sub-chan-

nels (ten liquid lines) and the use of a microprocessor to control the pneumatic pressure exerted on the tubes.
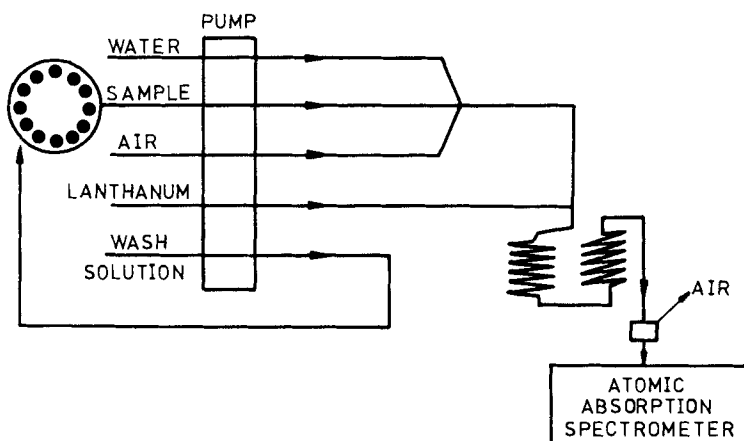


**Fig. 5.17** Continuous segmented analyser for atomic absorption spectrometric determinations. (Reproduced from [35] with permission of Perkin-Elmer).

(d) An air-pressure system synchronized with the pump left-off and controlled by an optical sensor which actuates solenoid valves in-phase with the pump rollers.

(e) The small bore of the transport and reaction tubes (1.0 mm), intended to avoid carry-over, increase the sampling frequency (120–240 h⁻¹) and lower the sample and reagent consumption —this is the result of exploiting one of the advantages of FIA configurations (see Chapter 6).

(f) A dual-channel fibre optic detection system using a single light source. The flow-cells (path length 10–75 mm) are self-aligned with the optical beam via a keyed mounting slot in the colorimetric casting.

(g) The segmented analytical stream is passed through the flow-cell without debubbling and the parasitic signals from the bubbles are eliminated with the aid of computer software. Maintaining the continuity of segmentation in this fashion minimizes dispersion and results in increased sample throughput.

This type of analyser is basically intended for non-clinical applications such us the monitoring of water pollutants, the determination of assimilable elements in soils or industrial analyses.

For clinical applications, Technicon launched an air-segmented flow analyser, the CHEM-1, based on a novel philosophy called 'capsule chemistry technology' by its inventors [32]. This involves the use of an inert fluorocar-

bonated liquid of high viscosity and polymeric nature already employed by the
Technicon RA-1000 batch analyser described in Chapter 8. A thin film of this
liquid retained on the walls of a Teflon tube encapsulates the segmented flow,
thereby keeping its components from the tube walls. This avoids the typical
carry-over of bare glass tubing (see Fig. 5.3). The mere replacement of glass
with Teflon so that the air is in direct contact with the tube walls and
ensures adequate sweeping is unsatisfactory as bubbles are segmented unevenly
in this configuration. Conversely, in the capsular flow configuration (Fig.
5.18), the air bubbles act as total barriers in directly wetting the encapsu-
lating film, thus completely and efficiently separating the liquid segments
and avoiding carry-over.
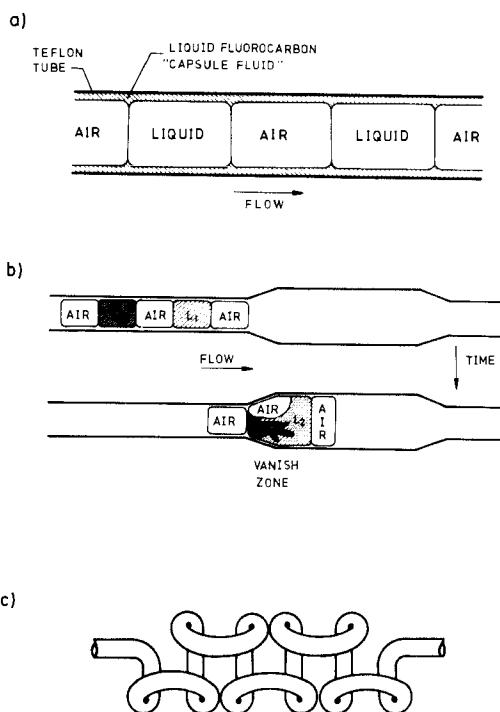


**Fig. 5.18** Innovative hydrodynamic principles of the CHEM-1 autoana-
lyser. (a) Capsule chemistry technology: the fluorocarbon liquid film
prevents contact between the segmented flow and the tube walls. (b)
Mixing of two liquid segments $L_1$ and $L_2$ separated by an air bubble in
the 'vanish zone'. (c) Special mixing loop. (Courtesy of Technicon).

Another novelty introduced with the CHEM-1 is the way in which sample and
reagents are mixed in the so-called 'vanish zone'. This is a widening of the
tube diameter by 50% (from 1 to 1.5 mm) over a short length, which causes a
momentary alteration in the flow: the separating air bubbles, of lower volume

than the liquid segments that they flank, detaches from the lower wall and al-
lows each pair of liquid segments to mix (Fig. 5.18b). A third innovative as-
pect of the CHEM-1 is the special design of its mixing loops (Fig. 5.18c), lo-
cated before and after the vanish zone, which increases the typical mixing ef-
ficiency of helical coils by a factor of 5.

These three innovations are associated with an extremely simple design of
the CHEM-1 hydrodynamic system which requires no carry-over suppressants or
additional merging or mixing units; in fact, a single-channel (567 cm in
length) manifold with a peristaltic pump is more than sufficient. Figure 5.19
shows the scheme of the essential elements of the CHEM-1, namely a sample and
reagent aspiration probe, a microconduit to the vanish zone, a series of photo-
metric sensors unevenly distributed along the tube and a peristaltic pump
which establishes the flow by aspiration.
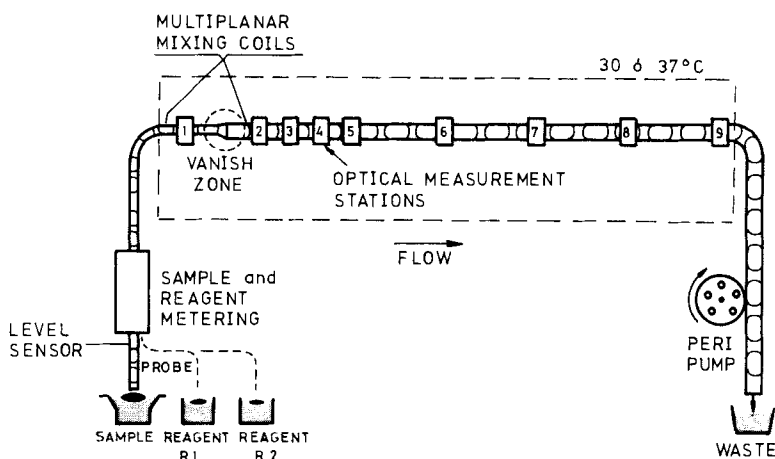


**Fig. 5.19** Scheme of the CHEM-1 segmented-flow analyser. (Courtesy of
Technicon).

Figure 5.20 depicts the sample and reagent introduction system, basically
a single Teflon probe of 1 mm ⌀ internally and externally wetted with the
fluorocarbon fluid. Each sample cup has a permanent floating drop introduced
before the determinations are started. This floating drop prevents contact be-
tween the different liquids as the aspiration probe is submerged in the cup
contents through it, thereby avoiding the use of a washing solution and in-
creasing the sample throughput. Sample and reagents are identified by means of
a bar code scanner, so they can be placed randomly on their respective trays.
The probe is initially introduced into each cup to withdraw a small volume of
the capsule fluid forming a film on its inner walls. Figure 5.20a shows the

situation of the probe after the sequential aspiration of sample-reagent 1 and reagent 2 with air bubbles of different volumes (test 1), and Fig. 5.20b depicts the scheme of the sample and reagent metering operation. Reagents and sample are introduced into the system by means of a series of four synchronized stop valves. During the sampling, stop valve 1 switches to allow sample aspiration through the 1-μL single-stroke, positive-displacement pump. As the stop valve closes, the probe is moved to a refrigerated (4-8°C) reagent carousel and positioned over the appropriate reagent cassette containing both reagents ($R_1$ and $R_2$) to be used in the selected test. The volumes of the reagents are measured with the aid of fifteen LEDs and matching optical detectors placed at heights that match probe contents of 7.0, 7.5, 8.0 and 8.5 μL. When the meniscus between $R_1$ and air is sensed at the appropriate height, the stop valves switch automatically to shut off further flow. The computer controlling the system also positions $R_2$ under the probe and introduces air and the water wash segments into the system. During operation, stop valve 4 connects the reagent and sample into the main channel. As the valve is open, the negative pressure produced by the single-tube peristaltic pump draws the appropriate
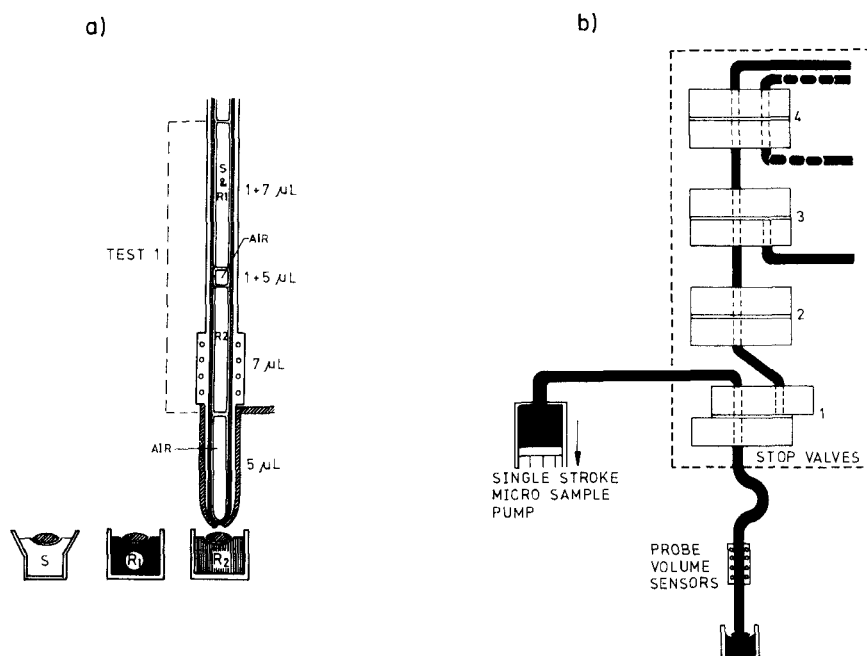


**Fig. 5.20** System for introduction of sample and reagents ($R_1$ and $R_2$) via a single probe into the CHEM-1 analyser. (a) Scheme of the segmented flow once introduced into the probe for a test. (b) Control of sample and reagent metering. (Courtesy of Technicon).

reagent past the probe volume sensor. When stop valve 4 is closed, an idle stream of air and water is aspirated into the analytical channel to maintain constant hydraulic conditions. Once the capsule has been completed, it passes into the temperature-controlled reaction chamber (Fig. 5.19) through a multi-planar coil which thoroughly mixes the contents of the first liquid segment, $R_1+S$, in the test capsule. This step initiates incubation of $R_1+S$ for about 5 min. The individual segments then pass through the first of a series of in-line detectors, also made of 1.0-mm I.D. Teflon. The absorbance of each liquid segment of the test capsule is read photometrically to establish the baseline reagent and serum absorbances for blank purposes. The test capsule then enters the vanish zone, where the segments are combined and the assay reaction is started. The test capsule, now a single 15-µL liquid segment, passes through a second multi-planar coil to ensure complete homogeneity of its contents. The diameter of the analytical pathway now decreases to 1.0 mm.

As shown in Fig. 5.19, the system has nine photometric detection stations based on the passage of a light beam through the main tube which allow data to be acquired from the segmented flow within 30 s after passage of a test capsule through the vanish zone, and subsequently at 30, 30, 30, 90, 90 and 90 s intervals (8 min altogether).

Each photometric station consists of three types of optical detector: bubble, spectrophotometric and nephelometric (see Fig. 5.21). The station first identifies a new test capsule by the unique absorbance pattern (at 800 nm) of the air-buffer-air segment preceding the capsule. Then it measures the absorbance of the passing test capsule: light from a central 56-W tungsten-halogen light source is passed through a rotating filter wheel containing eight filters. The collimated light is distributed to each of the in-line detectors by quartz optical fibres and then passed through the Teflon tubing and monitored by individual silicon photodiodes. Six successive absorbance readings of the central portion of the moving test capsule are taken at 100-ms intervals for all available wavelengths (340, 405, 500, 550, 570 and 600 nm) as the capsule passes the spectrophotometric detector. The signals are sent to an analogue/digital converter and transformed into concentration units by a series of algorithms. Then, the in-line detector monitors the light scatter of particles produced in the latex particle agglutination immunoassays and immunoprecipitation methods for specific proteins. When placed at a 30° angle to the light source, the detector functions as a nephelometric detector. A separate light source and filter wheel are used for this application; particle formation is monitored at a 400–500 nm bandwidth for the immunoprecipitation methods and 800–1000 nm for latex agglutination to obtain the maximum signal.

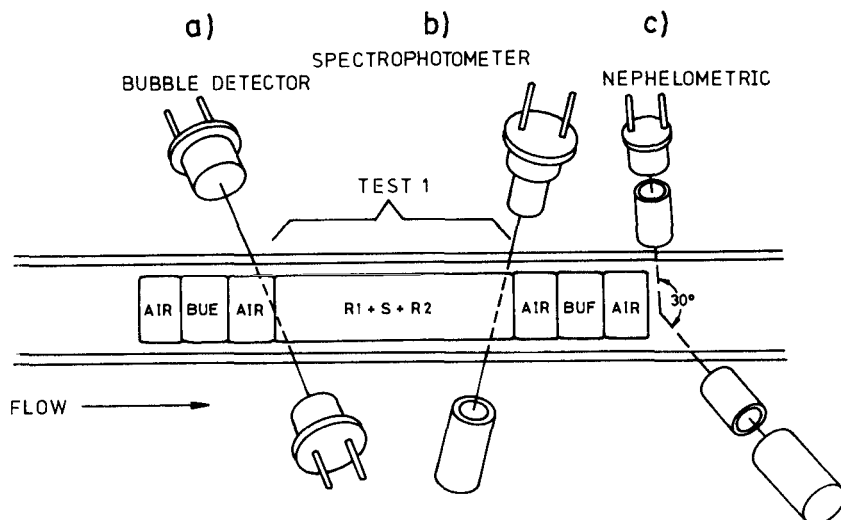The CHEM-1 is thus a sophisticated analyser requiring strict control of

**Fig. 5.21** Photometric detection unit of the CHEM-1 featuring (a) bubble, (b) spectrophotometric and (c) nephelometric optical detectors. (Courtesy of Technicon).

functioning and self-regulation by a computer which also acquires and processes data and delivers results as requested. It uses samples and reagents fairly sparingly and allows up to 35 different methodologies (both end-point and kinetic) to be implemented at a rate of up to 1800 parameters per hour. It is able to perform urgent determinations without essentially altering its programmed functioning. By use of an additional module, consisting of three selective electrodes plus a reference electrode, it allows the determination of sodium, potassium and carbon dioxide. The module probe takes sample aliquots and introduces them into a vessel where they are diluted with the aid of the probe itself. The vessel contents are then drained and sent to the potentiometric multidetection system for sensing.

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# 6
## Automatic continuous analysers. II.
## Flow-injection analysis

### 6.1 INTRODUCTION

Flow-injection analysis (FIA) falls clearly into the classification of automatic continuous methods of analysis established in the preceding chapter. This novel technique features a simple basis, inexpensive labware, and straightforward and convenient operation. In addition, and most surprising taking into account all the aforesaid virtues, it provides excellent results. Its extreme versatility singles it out from most of the more recent analytical techniques. On the one hand, it can be readily adapted to virtually every type of analytical problem, and on the other, it allows for direct control by the researcher. Other salient features of FIA are as follows:

(a) The flow is not segmented by air bubbles, which marks an essential difference between this methodology and segmented flow analysis, SFA.

(b) The sample, a liquid, is injected or inserted directly into the flow rather than aspirated into it.

(c) The injected plug is transported along the system. This physical process may be concurrent with others of a different nature (chemical reaction, dialysis, liquid-liquid extraction, etc).

(d) The extent of dispersion or dilution undergone by the analyte in the above-mentioned transport process can be readily controlled through the geometric and hydrodynamic characteristics of the system. Sample and reagent(s) mix incompletely, although reproducibly, giving rise to a concentration gradient which varies along the system as a function of time.

(e) The continuous detector usually employed provides a transient signal which is suitably recorded.

(f) Neither physical equilibrium (homogenization of a portion of the flow) nor chemical equilibrium (reaction completeness) has been attained by the time the signal is detected. Hence, FIA can be considered a fixed-time analytical methodology.

(g) The operational timing must be highly reproducible as measurements are carried out under non-steady-state conditions, so that small changes may give rise to serious modifications of the results obtained.

All these features can be combined into four wider aspects: *unsegmented flow, direct injection, controlled partial dispersion* and *reproducible operational timing.*

Figure 6.1a shows a basic FIA configuration consisting of: (a) a propelling unit, which drives one or several flowing stream solutions (either containing a dissolved analyte or acting as mere carriers) at a constant rate; (b) an injection system which allows the reproducible insertion or introduction of an accurately measured sample volume into the flow without halting it; (c) a piece of tubing, usually referred to as reactor, along which the reacting mixture is transported; (d) a flow-cell accommodated in a measuring device (photometer, fluorimeter, potentiometer) which transduces the signal generated to a recorder and/or microprocessor —usually, the stream emerging from the sensing system is wasted.
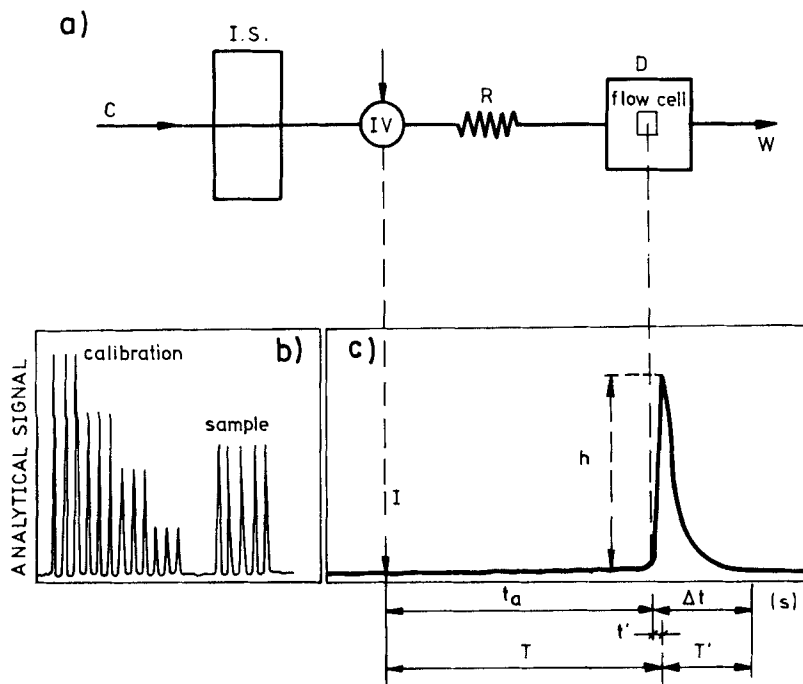


**Fig. 6.1** (a) Scheme of a basic FIA system. C: carrier (with or without a dissolved reagent); PS: propelling system; IV: injection valve; R: reactor; D: detector; W: waste. (b) Slow recording —the commonest in this technique— corresponding to injection of triplicate standards and quintuplicate samples. (c) Fast recording, with its characteristic parameters.

Figures 6.1b and c show typical recordings obtained with this technique

(analytical signal *vs.* time). The parameters defining such recordings are as follows:

(a) *Peak height*, h, related to the analyte concentration.

(b) *Residence time*, T, defined as the interval elapsed from injection to the appearance of the maximum signal.

(c) *Travel time*, $t_a$, namely the period separating injection from the end of the baseline.

(d) *Return time*, T', *viz.* the time elapsed between appearance of the maximum signal and baseline restoration.

(e) *Baseline-to-baseline time*, $\triangle t$, or interval elapsed between the onset of the peak and baseline restoration.

It should be noted that FIA peaks are in no way Gaussian curves, so that none of the parameters above describes the peak shape in full —particularly the trailing portion, which is peculiar of this type of recording and distinguishes it from the transient signals typical of other analytical techniques.

A comparison between the basic features of FIA and SFA (Fig. 6.2) allows the following essential differences to be established:
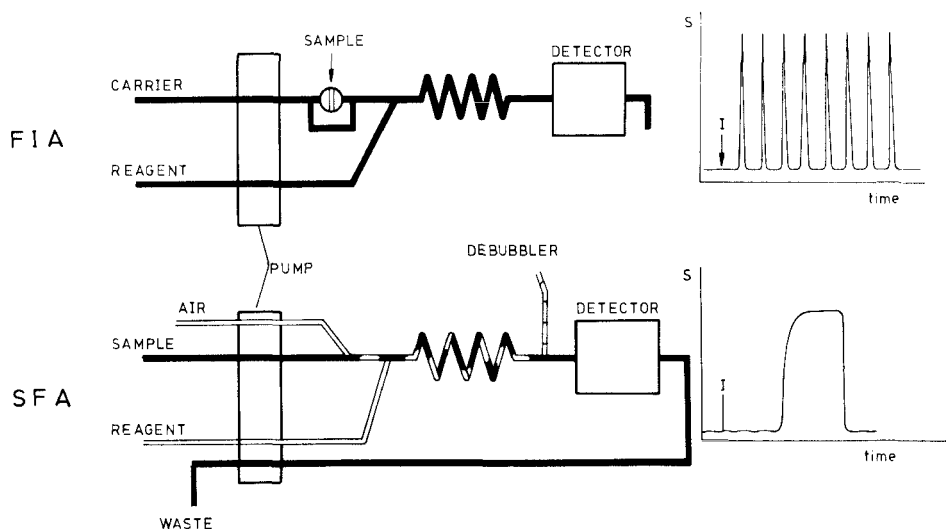


**Fig. 6.2** Comparison between FIA and SFA systems and their characteristic recordings. The chief difference lies in the use of bubbles in SFA. The passage of the flowing stream emerging from the detector through the pump is intended to regulate the flow-rate more accurately in order to achieve reproducible splitting of the segmented flow.

*Air bubbles.* Their presence in the flow is the most significant differ-ence between both methodologies. While SFA relies on flow segmentation, the most significant contribution of FIA is the absence of bubbles and hence the avoidance of their inherent shortcomings, namely impracticability of miniatur-ization, pulsations due to the different compressibility of liquids and gases, decreased efficiency of the continuous separation devices incorporated in the system, generation of static electricity, irreproducibility in the implementa-tion of stopped-flow kinetic methods, difficulty in controlling the flow-rate and technical complications arising from the incorporation of bubblers and debubblers. All these drawbacks, from which FIA is completely free, make this methodology a major analytical alternative with the additional advantages of perfectly reproducible flow-rates, simple and inexpensive labware, miniaturiza-tion capability, high sample throughput, great analytical potential and broad scope of application.

*Technical features.* Notwithstanding their instrumental likeness —both methodologies make use of similar reactors and propelling systems—, there are a number of essential differences between the two, the most marked of which are as follows:

(a) The start-up time is longer in SFA, where it is usually necessary to wait between 30 and 45 min in order to obtain reliable measurements. The only delay time common to both this and FIA is that required for the detector stab-ilization.

(b) The geometric characteristics of the manifold are different. The tub-ing diameter is usually much smaller in FIA, which determines the physical behaviour of the injected plug.

(c) The flow-rates commonly employed in FIA are normally lower than those used in SFA.

(d) The sample is aspirated in SFA and either injected or inserted direct-ly into the flow in FIA, which calls for different labware.

(e) The sampling time is longer and the sample volume is larger in SFA.

(f) The wash cycle, essential in SFA to avoid carry-over in both sampling and transport, is unnecessary in FIA.

*Detection.* Despite the fact that both methodologies use very similar sens-ing systems, there is a basic difference as regards the moment at which detec-tion is performed. Thus, in SFA the signal is measured under steady conditions (when at least 95% of the maximum signal has been attained) and the plug pass-ing through the detector is virtually homogeneous, so that the recordings ob-tained are plateau-shaped (Fig. 6.2, bottom). In contrast, FIA measurements are carried out under non-steady conditions on non-homogeneous plugs (Fig. 6.2, top).

*Versatility.* Flow-injection analysis is much more versatile than segmented-flow analysis; the former allows for the implementation of a variety of techniques (titrations, stopped-flow methods, etc) which are inapplicable with the latter.

*Data supply.* The unsegmented mode clearly exceeds SFA in its information capability as, in addition to the peak height (common to both), it provides interesting data such as peak areas, peak widths (in the gradient mode) and peak-to-peak parameters (in the stopped-flow variant).

Table 6.1 compares SFA in FIA in terms of the above characteristics.

**TABLE 6.1**

Comparison between the two chief types of continuous analytical methodologies: segmented (SFA) and unsegmented (FIA)

| Feature | SFA | FIA |
|---------|-----|-----|
| Sample introduction | Aspiration | Injection |
| Sample volume | 0.2-2 mL | 10-100 µL |
| Response time | 2-30 min | 3-60 s |
| Tubing inner diameter | 2 mm | 0.5-0.7 mm |
| Detection | In equilibrium (homogeneity) | With controlled partial dispersion |
| Sample throughput | $\ll$80 samples/h | $\ll$300 samples/h |
| Precision | 1-2% | 1-2% |
| Reagent consumption | High | Low |
| Washout cycle | Essential | None |
| Continuous kinetic analysis | Not feasible | Stopped-flow |
| Titrations | No | Yes |
| Data supplied | Peak height | Peak height, area and width; peak-to-peak distance |

## 6.2 FOUNDATION OF FLOW-INJECTION ANALYSIS

The physical foundation of FIA is related to the behaviour of the sample plug inserted in the flow, which is characterized mathematically by means of the so-called *dispersion*. This, in turn, is defined by the shape of the profile yielded by the injected sample portion along the system —particularly at the flow-cell.

Although the nil mixing between successively injected samples was formerly attributed to the turbulent regime under which FIA experiments were believed to be conducted [1,2], it has been demonstrated beyond doubt [3,4] that most FIA systems operate under laminar flow conditions (Fig. 6.3).

## CONVECTIVE TRANSPORT

TURBULENT FLOW          LAMINAR FLOW

## DIFFUSIONAL TRANSPORT

AXIAL          RADIAL
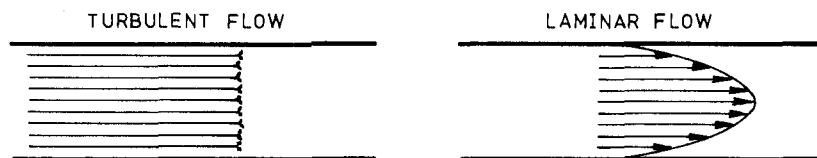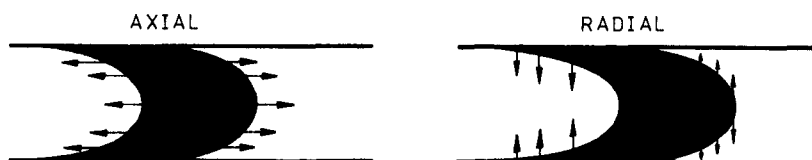
**Fig. 6.3** General types of transport in closed tubes.

When the sample is injected into the flowing stream, its dilution within it is a function, of, among other factors, the time considered along the transport process. Initially, dilution is chiefly convective in nature; later it becomes convective-diffusional and eventually develops a purely diffusional character. The intermediate situation (i.e. convective-diffusional transport) is by far the most common in FIA.

It has been demonstrated that radial dispersion contributes more significantly to the dilution of the sample in the flow than does axial dispersion. This type of fluid movement, termed 'secondary flow' by Tijssen [4], results in a washout effect accounting for the low mutual contamination of samples successively injected into a carrier stream. This advantageous feature is a result of the use of low flow-rates and small tubing bores, and results in decreased peak-width and hence to increased sampling rate.

Theoretical studies on FIA have been aimed at the establishment of accurate relationships between the geometric (length and diameter) and hydrodynamic (flow-rate) characteristics of an FIA system and the parameters defining the profile obtained (travel time, coordinates of the peak maximum and peak width).

Several models have been proposed to account for the non-Gaussian shape of

typical FIA recordings, which reflects the odd behaviour of a solute injected into an FIA system. The best known among such models are the following:

*Taylor's model.* This is applicable only when purely diffusional or convective phenomena are involved and is therefore based on a Gaussian conception of the FIA curve.

*Tanks-in-series model.* This model is based on the assumption that the system is composed of n perfect minimixing chambers resembling chromatographic plates, and its accuracy increases with increase in the number of plates considered. In any case, it describes more factually ordinary FIA systems than does Taylor's model.

*Mixing chamber model.* This is valid only when a perfectly stirred mixing chamber is used.

*General model.* This is the best alternative as it describes in the most accurate way possible the behaviour of a solute injected into an FIA system. It is based on the general expression describing convective-diffusional transport, which takes account of both axial and radial concentration gradients, the parabolic shape of the velocity profile corresponding to a laminar flow regime and the contribution of convective transport

$$D \left[ \frac{\delta^2 C}{\delta x^2} + \frac{\delta^2 C}{\delta r^2} + \frac{1}{r} \frac{\delta C}{\delta r} \right] = \frac{\delta C}{\delta t} + u_0 \left[ 1 - \frac{r^2}{a^2} \right] \frac{\delta C}{\delta x} \qquad (6.1)$$

where D is the molecular diffusion coefficient (expressed in $cm^2/s$), C is the analyte concentration, t is the time (in s), x is the distance from the tube radius (cm) and $u_0$ is the linear flow velocity (cm/s) [5].

Equation 6.1 is by no means easy to solve in a straightforward manner. By use of suitable approximations, Vanderslice *et al.* [3] derived two expressions relating two of the most relevant parameters of the FIA curve (travel time and peak width) to the essential characteristics of an ordinary FIA system (*viz.* the reactor length, l, and radius, r, and the flow-rate, q), together with the molecular diffusion coefficient:

$$t_a = 109 r^2 D^{0.025} (L/q)^{1.025} (1/f) \qquad (6.2)$$
$$\Delta t = 35.4 (r^2/D^{0.36})(L/q)^{0.64} f \qquad (6.3)$$

These two expressions are descriptive of experimental facts only if an *accommodation factor*, f, is included.

A different approach to the problem is represented by Ruzicka and Hansen's *dispersion coefficient*, D, defined as the ratio between the analyte concentration prior to and after the transport process [6]. It coincides with the ordinate at the maximum of the FIA curve and is related to other parameters through the expression [7]

$$D = 2\pi^{3/2}r^2L^{1/2}u^{1/2}\delta^{1/2}t_r^{1/2}V_i^{-1} \tag{6.4}$$

where $t_r$ is the statistical residence time, $V_i$ is the injected volume and $\delta$ is the Levenspiel–Smith linear dispersion number, which in turn is related to the standard deviation of the curve by

$$\delta = (1/8)[8(\sigma^2 + 1)^{1/2} - 1] \tag{6.5}$$

This complicates the practical application of the model because of the non-Gaussian shape of the FIA curve, which must unavoidably be traced in order to determine its exact profile.

Equations (6.2) to (6.4) provide both 'horizontal' and 'vertical' information on the FIA curve, but lack practical and direct application owing to the introduction of the accommodation factor in the first two and that of the linear dispersion number in the third.

A few experiments and a suitable computational program [8–10] recently allowed several expressions relating the characteristic parameters of the FIA peak to those representative of the FIA system to be obtained:
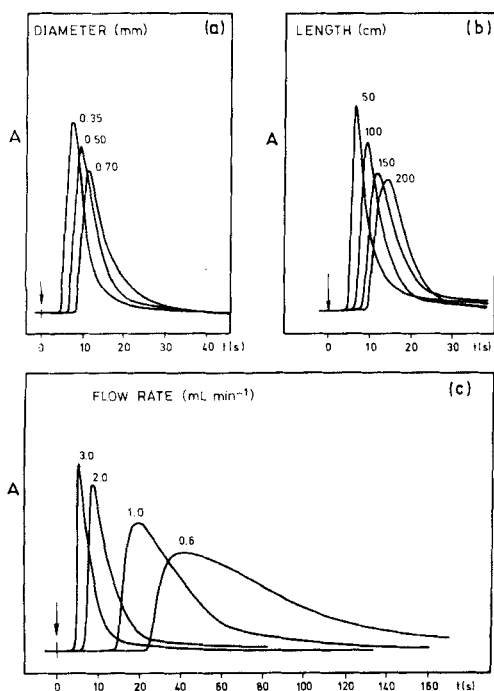


**Fig. 6.4** Influence of FIA variables on the profile of the FIA recording. (a) Effect of the reactor diameter (L = 100 cm; q = 2.0 mL/min). (b) Influence of the reactor length ($\emptyset$ = 0.5 mm; q = 2.0 mL/min). (c) Influence of the flow-rate (L = 100 cm; $\emptyset$ = 0.5 mm).

$$t_a = 0.465 \ d^{0.150} \ L^{0.850} \ q^{-0.850} \tag{6.6}$$
$$\Delta t = 56.747 \ d^{0.283} \ L^{0.107} \ q^{-1.057} \tag{6.7}$$
$$D = 2.342 \ d^{0.496} \ L^{0.167} \ q^{-0.206} \tag{6.8}$$
$$T = 0.840 \ d^{0.683} \ L^{0.801} \ q^{-0.977} \tag{6.9}$$

Figure 6.4 shows several FIA curves obtained with an ordinary FIA setup. As can be observed, the dispersion increases with increasing reactor diameter (a) and length (b), and decreases with increase in the flow-rate (c). The extent of dilution or dispersion undergone by the analyte also depends on several other factors such as the injected volume and dead volumes in the connectors or in the flow-cell.

All the statements made so far are valid only in the absence of a chemical reaction. If such a reaction is involved, it is necessary to take into account all its kinetic aspects as these affect the characteristics of the FIA peak considerably. Painton and Mottola [11] evaluated the kinetic contribution ($D_c$) to the dispersion coefficient due to physical phenomena, finding that the expression $D = D_c + D_p$ holds provided that the chemical reaction results in a decrease in the concentration of the monitored species.

More recently, Painton and Mottola have gone deeper into the study of the chemical contribution to the dispersion [12] by checking the suitability of introducing a term of the form $-k.C^n$ into the left-hand side of the general equation governing diffusional transport. Such a term represents the extent of dilution undergone by the sample plug upon chemical reaction.

## 6.3 BASIC COMPONENTS OF AN FIA SYSTEM

An ordinary FIA system usually consists of a least four essential parts:

(a) A *propelling system*, which drives the carrier stream to the different elementary units of the system. Ideally, it should provide a pulse-free and perfectly reproducible flow of constant rate.

(b) An *injection* or *insertion system* for introduction of variable sample volumes into the carrier stream in a highly accurate and reproducible manner.

(c) A *transport system* linking the various elements which make up the FIA system and allowing the sample to attain a suitable degree of dispersion or mixing as it travels through it. When the extent of dispersion is not suitable for the experiment concerned and a reaction or further splitting of the flowing stream is required, the system can be supplemented with accessories such as mixing chambers, reactors and merging points.

(d) A *sensing system* allowing continuous monitoring of a given property of the sample or its reaction product and providing qualitative and quantitative information about the former.

### 6.3.1 Propelling system

On account of the constancy of the flow-rate provided, undoubtedly the most important prerequisite to be met, the peristaltic pump is the fluid propelling system most frequently used in FIA. Other lesser common alternatives are gas pressure and gravity pressure-based devices.

Peristaltic pumps call for the use of flexible tubing squeezed by a set of rollers whereby the fluid enclosed in the system is kept in motion. This type of pump provides a pulsed flow, which is undesirable in FIA. The pulse duration, which decreases with the number of rollers used and decreases with their diameter, dictates the number of tubes (lines) through which the fluids involved can be propelled (between 1 and 16, but 4 in most cases).

Pressure-based propulsion, whether by gravity or by a gas, results in pulse-free flow, but poses serious problems in controlling the flow rate of two or more simultaneous streams.

### 6.3.2 Injection system

The injection system is intended to place a well-defined sample plug into the flowing carrier stream and should meet a number of requirements imposed by the intrinsic features of the FIA technique, namely:

(a) It should insert accurately measured volumes of sample in a reproducible manner. The wider the volume range is, the more versatile the system will be.

(b) The sample should be incorporated into the carrier stream in such a way that no disturbances to the latter are caused.

(c) It should allow for convenient and fast operation in order to achieve a high sampling rate. In addition, it should be controllable by an electric motor.

These requirements make the aspiration systems commonly employed in SFA unusable in FIA. In fact, such systems result in relatively inaccurate sampling, with variations in the injected sample volumes of the order of 3-4%, which does not represent a serious problem in SFA, in which the indicator reaction does attain equilibrium and volume variations affect the peak width rather than the peak height, which is the parameter of actual analytical interest.

The earliest injection unit employed in FIA was as simple as a syringe and hypodermic needle. Currently, the injection systems most frequently used are the rotary valve and the proportional injector.

The so-called four-way or hexagonal rotary valve (Fig. 6.5a) consists of six ports (three inlets and three oulets) and can adopt two positions. In the filling position, the sample enters the valve through port 2, fills the sample

loop (ports 1 and 4) and is continuously wasted through port 3; meanwhile, the carrier stream goes into the valve through port 6 and leaves it through port 5 in the direction of the reactor. In the evacuation position, port 6 is linked internally with port 1 and the carrier sweeps the sample, which enters the valve through port 2 and is wasted through port 2, towards the reactor via ports 4 and 5.
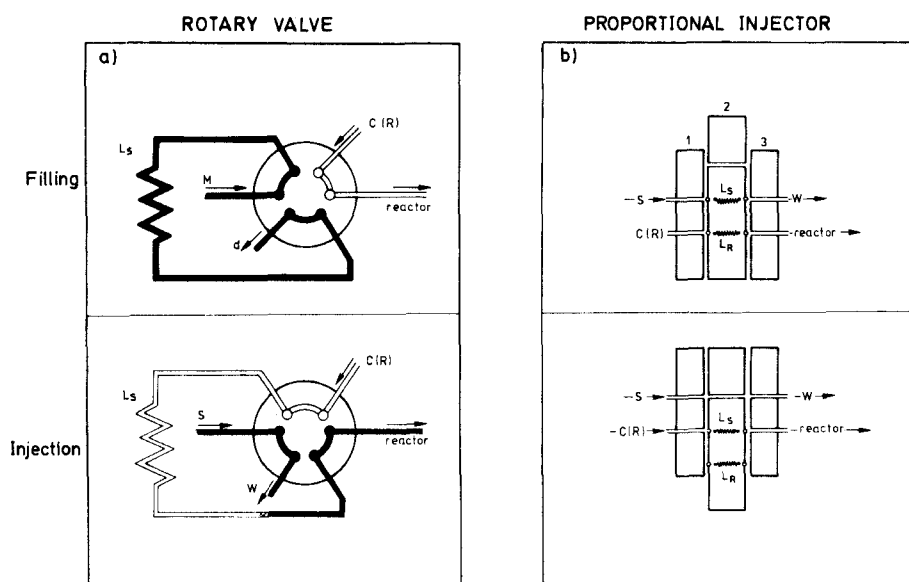


**Fig. 6.5** Schemes of typical injection systems in their filling and evacuation positions. (a) Rotary valve; (b) proportional injector.

The proportional injector, developed by Bergamin *et al.* [13], has been improved since its creation to adapt it to different FIA modes. The simplest version of this type of injection system (Fig. 6.5b) consists of three drilled polyethylene or Perspex blocks (two fixed and one moving). In the filling position, the upper and lower ports of each block are matched so that the sample (upper port) enters through block 1, fills the loop in block 2 and runs to waste through block 3; the reagent or carrier solution enters through the lower port of block 1 and leaves towards the reactor via block 3. In the evacuation position, the central block rises until its lower port (sample) confronts the lower ports of the side blocks, so that the sample solution held in

the corresponding loop (Ls) is swept to the reactor by the reagent or carrier stream.

Both the rotary valve and the proportional injector supply highly reproducible, variable  injected volumes (held in loops) and are fast and convenient to operate manually. In addition, they allow for easy automation of the injection operation.

### 6.3.3 Transport and reaction system

In addition to its primary function of transporting the flowing stream along the manifold and allowing, where appropriate, a reaction to develop to a suitable extent, the transport and reaction system serves to link the different parts of the FIA system.

The transport system normally consists of small-bore tubes of I.D. between 0.1 and 2 mm, but usually 0.3, 0.5 and 0.7 mm for low, medium and high dispersion requirements, respectively. Larger diameters are of little use as the remainder of the FIA components are not designed to match them —tubing expansions and compressions of other components result in irregularities that significantly affect the dispersion. Diameters smaller than 0.3 mm (e.g. 0.2 mm) are used only in the miniaturized FIA mode, *viz.* capillary FIA. Teflon, polyethylene and polypropylene are the materials most commonly used in the manufacture of FIA tubing. As all three are highly permeable to oxygen, serious problems are encountered when dealing with strong reductants. On the other hand, they are of great use in checking for catalysed aerial oxidations.

The connectors used in an FIA set-up serve the purpose of joining the tubes to one another and to the other parts of the system. There is a wide range of connectors available for each type of application, but basically they are either dual (linear or V-shaped), triple (T-, Y- or W-shaped) or quadruple (usually in the shape of an arrowhead).

The reactor, a major component of the transport system, influences the residence time and the profile of the sample plug, and is designed to meet the particular needs of the system concerned. There are five basic types of FIA reactor:

*Open tubes.* These are straight  tubes of variable length and diameter, located between the injection and sensing systems.

*Coils* are pieces of tubing helically coiled around a rigid cylinder of the desired diameter.

*Packed reactors* usually consist of a length of tubing filled with a chemically active or inert material. They have not been widely used in FIA so far as packing materials with large particle sizes give rise to insurmountable irregularities in the flow, while small-sized particles result in increased re-

actor compactness and hence in greater flow resistance, which calls for the use of high pressures, thus detracting from the inherent simplicity of the FIA technique. Chemically active packed reactors (ion-exchange, redox and enzyme) have been preferentially used over inert reactors so far.

The *single bead string reactor* (SBSR) is essentially a piece of tubing of variable length packed with glass or chemically inert polymer beads whose diameter is 60-80% that of the tubing, so that the tube cross-section is occupied by only one bead at a time. As the packing does not take up much space, the flow encounters little resistance. The use of this type of detector offers advantages such as increased residence times, ostensibly decreased dispersion and smooth baselines.

*Mixing chambers* are used when a high degree of mixing (merging point or unstirred chamber) or complete homogenization of sample and  reagent (well-stirred chamber) is required, which is usually the case when using an electric system or when there are considerable differences in viscosity, specific gravity, temperature or detergent content between the carrier and sample [15]. They are also commonplace in FIA titrations.

### 6.3.4 Sensing system

Detectors to be used in FIA should ideally be endowed with a number of attributes such as low flow-cell volume and noise, flow-rate-independent signal, fast and linear response over a wide concentration range and high sensitivity. FIA methodology utilizes a variety of analytical detection techniques such as optical (spectroscopic and non-spectroscopic), electric (amperometric, potentiometric, conductimetric, coulometric) and thermochemical.

The use of the voltammetric technique has fostered the development of a host of cells and electrodes, both solid and liquid (DME) [16-25], as well as the study of the principles describing the behaviour of such devices as parts of FIA systems [23-25].

Redox [26], ion-selective [27-30] and ISFET [31-32] potentiometric sensors have been widely used in FIA applications. In contrast, voltammetric [33,34] and potentiometric [35,36] stripping, coulometry [37,38] and conductimetry [39-41] have received less attention from FIA researchers.

Optical detectors  are by far the most common in FIA, even though  only a few dedicated cells have been designed as most of the cells, particularly photometric and fluorimetric, manufactured for other flow methods can be readily adapted for use in FIA. On the other hand, other optical techniques such as chemiluminescence [42] and refractometry [43], of more limited use in this methodology, have favoured the development of new types of cell suited to specific  needs. At this point it is worth noting the simplicity of the joint
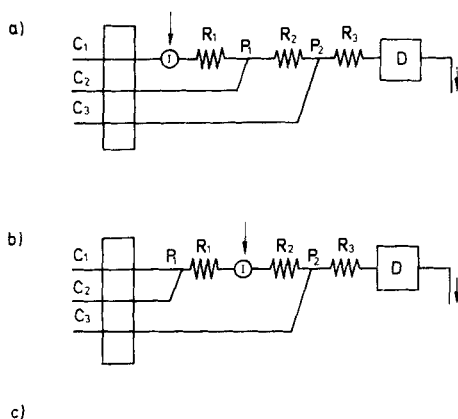
use of FIA and atomic techniques; in fact, no sensing cell is required as the injected sample is directly aspirated into the burner [44].

It is significant that despite the large number of FIA applications developed since the introduction of this methodology in 1975 [1,45], not many commercial instruments have been designed in this field, probably because of the hindrances posed by the companies devoted to the manufacture of batch and segmented flow analysers, which are usually much more expensive and complicated than FIA instruments. This initial reluctance is gradually being overcome and there are already a number of companies in the USA, Brazil, Sweden and Japan constructing and commercializing various types of FIA autoanalysers.

## 6.4 FIA MODES

One of the most prominent features of FIA is its great versatility, irrefutable proof of which is the large number of alternatives deriving from the basic mode and developed for specific purposes. Among the large variety of FIA



**Fig. 6.6** Different types of primary FIA configurations. (a) With both merging points situated after the injection system; (b) with one merging point located prior to and another after the injection unit; (c) classification of primary FIA configurations according to the number of channels, reagents, coils and merging points used.

modes available to date, eight of them are worth special note, namely primary FIA, reversed FIA, FIA in closed systems, zone manipulation, miniaturized FIA, gradient FIA, kinetic methods developed by FIA and FIA associated with separation techniques.

*Ordinary* or *primary FIA* involves a single injection of the sample, which is inserted into a continuously flowing carrier with which it can react or not. The reaction(s) can also be induced by merging one or several suitable streams with the main line. No second phase is necessary for the reactions involved (redox, complex formation, etc.) to occur. The transient signal generated is always measured at the point of maximum reaction development. The two most general types of primary FIA configuration with one, two and three channels, depending on the relative situation of the merging points with respect to the injection unit, are depicted schematically in Fig. 6.6. Four and five-channel primary FIA systems have also been used at times [46] in dealing with complex chemical systems in which the formation of the monitored product involves a large number of steps or with very complicated samples calling for various masking operations.

In *reversed FIA* the sample is circulated along the main line of the manifold, into which the reagent(s) is (are) injected [47,48]. The basic differences between this mode and primary FIA are illustrated in Fig. 6.7. Reversed FIA is of great use when very frequent analyses of abundant and inexpensive samples (e.g. in the monitoring of pollution in waste waters) [49–51] or increased sensitivity [52] are required. The only two disadvantages of this mode are its low sampling frequency and the considerable sample consumption. Sample changeovers entail washing the system with the actual sample, which results in
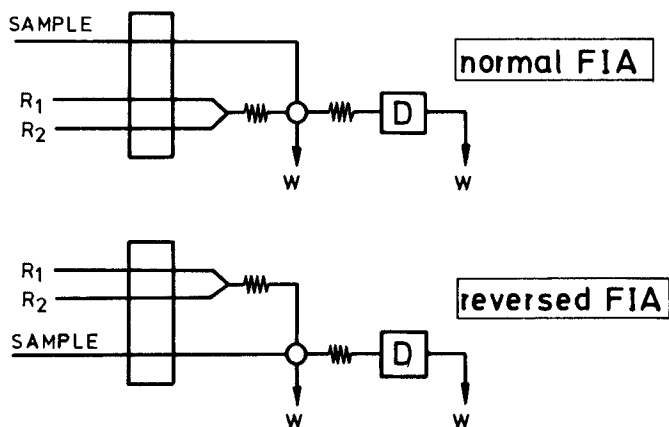


**Fig. 6.7** Comparison between normal and reversed FIA set-ups.

a delay period whose length is proportional to the difference in concentration between successively injected samples. The continuous circulation of the sample along the system results in increased consumption in comparison with normal FIA.

Flow-injection analysis can be implemented with the aid of closed systems of two basic types: closed-loop and closed-open systems.

In *closed-loop systems* the flow is continuously recirculated along the system and a purpose-built unit situated after the sensing system regenerates and/or removes certain species. These systems are particularly suitable for reactions involving dissolved enzymes [53,54]. A representative example is illustrated in Fig. 6.8 for the catalytic determination of copper with the aid of the indicator system $Fe^{3+}/S_2O_3^{2-}$. The uncatalysed reaction is not sufficiently slow to give rise to a steady baseline. This shortcoming is circumvented by injecting one of the reactants (thiosulphate) simultaneously with the sample instead of incorporating it into the solution reservoir. The rate of the overall reaction (catalysed + uncatalysed) is monitored absorptiometrically by addition of thiocyanate, with which iron forms the red complex $Fe(H_2O)_5SCN^{2+}$. The formation constants of the iron complexes (roughly 100 for $Fe^{3+}$ and 10 for $Fe^{2+}$) allow for the occurrence of sufficient free $Fe^{2+}$ to give rise to the anodic oxidation of this species at a controlled potential and hence for the regeneration of both $Fe^{3+}$ and the monitored species. Such an oxidation reaction can be conveniently carried out at a potential of +0.60 V *vs.* SCE and allows the separation of catalytic copper ion through cathodic reduction to metal copper and hence isolation of the catalyst and regeneration
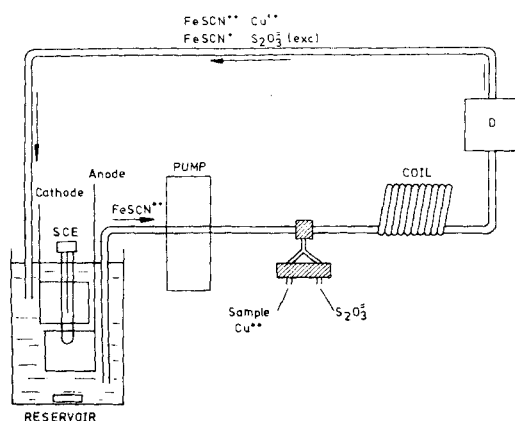


**Fig. 6.8** Closed system for the catalytic photometric determination of copper using the $Fe^{3+}/S_2O_3^{2-}$ system and thiocyanate as a 'revealer', featuring a special unit for removal and regeneration of species. (Reproduced from [54] with permission of the American Chemical Society.)

of the main reactant of the indicator reaction simultaneously. The electro-
lytic process further decreases the rate of the uncatalysed reaction as thio-
sulphate decomposes to gaseous $SO_2$ in the electrochemical cell [55].

Figure 6.9 depicts a typical closed–open system, whose key element is a
selecting valve (SV). This allows the system to be kept open until the sample
reaches the inner circuit. By appropriately switching the valve, the sample is
allowed to circulate through the loop until completely homogenized with the
carrier, which entails n passages through the circuit and results in the acqui-
sition of n signals at the detector. The curve recorded by monitoring the dis-
appearance of a reactant (a) or the formation of a product (b) —rather unchar-
acteristic in either case— has the typical shape of a classical kinetic curve
and therefore allows the calculation of the rate of the reaction involved,
application of amplification or dilution methods (based on the sum of the
height of several peaks and on the measurement of peaks other than the first
and thus lower than it), kinetic monitoring of a single species [56], applica-
tion of differential kinetic methods [57], calculation of stoichiometries
[58], speciation [59] and so forth.
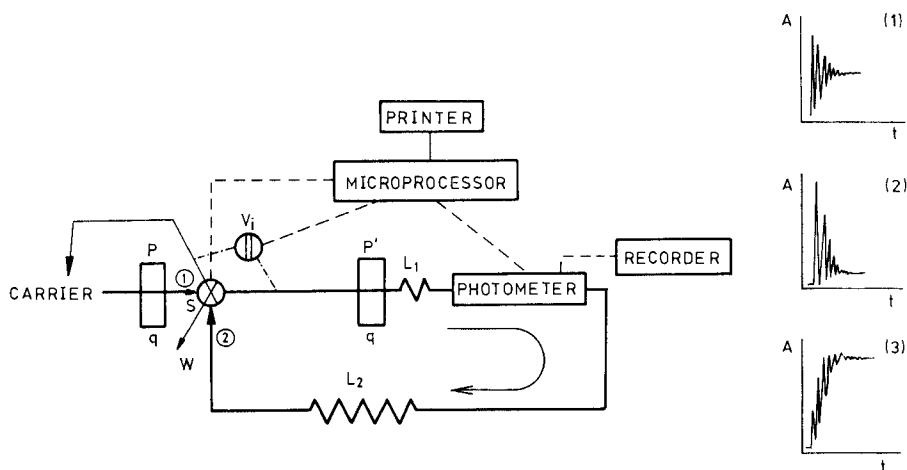


**Fig. 6.9** Closed–open system for multi-detection and typical record-
ings obtained by (1) injecting a dye, (2) monitoring the disappearance
of a reactant and (3) monitoring the formation of a product. The in-
jection valve, $V_i$, can be placed inside or outside the loop. S denotes
a selecting valve which can be switched either to allow the flowing
stream to enter the loop or to be trapped within it and be kept recir-
culating by means of pump P until complete homogenization is achieved
—in this position, the carrier is sent directly to waste. (Reproduced
from [56] with permission of the American Chemical Society).

The sample, or indeed a portion of it, can be advantageously manipulated
in a variety of ways to improve the features of conventional FIA. The   so-

called *zone manipulation* mode has given rise to three different submodes: merging zones, zone sampling and zone trapping.

(a) The *merging zones mode* is of enormous interest when minimum sample and reagent consumption is desired (e.g. in enzymatic clinical analyses). It involves (Fig. 6.10) the use of two channels of the same length which converge on a point prior to which is located a dual injection valve inserting low sample and reagent volumes into their corresponding lines. On merging, the two plugs mix thoroughly, which facilitates reaction development [60,61].
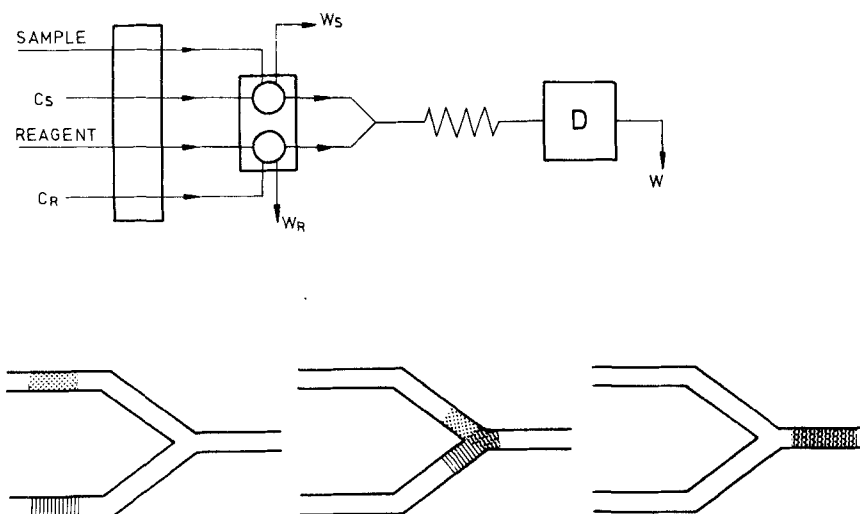


**Fig. 6.10** Typical set-up for implementation of the merging zones mode. The two loops of the dual injector are loaded with sample and reagent, which are subsequently injected simultaneously into channels of the same geometric characteristics that merge elsewhere in the system.

(b) The *zone sampling* mode is implemented by introducing a preselected portion of the sample into a second carrier stream [62], thereby obtaining two sample subplugs of different concentration which can be used to determine two species requiring different degrees of dispersion [63].

(c) The so-called *zone trapping* mode, a variant of zone sampling, involves separating the reacting plug from the flowing stream by means of a suitable device (usually a proportional injector) over the time needed for the

reaction to develop to an appropriate extent with no increase in dispersion. Once such a period has elapsed, the trapped plug is returned to the circulating stream, which drives it to the detector [64].

As with other analytical techniques, there is a trend in FIA towards significant reductions in size, which ultimately result in considerable advantages. In this way *miniaturized FIA* in its two versions was conceived: capillary FIA and integrated microconduits.

(a) The tubes used in *capillary FIA* rarely exceed 0.2 mm in diameter and 1 μL in volume. Such small dimensions enable the experimenter to obtain significantly increased sampling rates with sparing sample and reagent consumption [65,66].

(b) *Integrated microconduits* are a relatively recent conception of FIA assemblies consisting of a series of sinusoidal channels engraved on a PVC block of 70 x 45 x 10 mm and covered with a thin lid of the same material in such a manner that the microconduit section is semicircular. The block can accommodate (Fig. 6.11) an injection unit (hydrodynamic injection system [64]), a sensing system (photometric probe, selective electrode) and/or a separation unit (dialysis membrane, ion-exchange column) [67,68]. The rigidity of the structure ensures perfect reproducibility in the measurements.
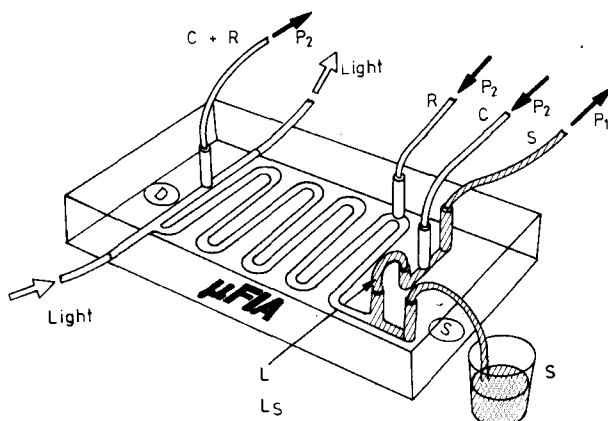


**Fig. 6.11** Integrated microconduits. The sample is aspirated into its corresponding loop, Ls (20 μL) through pump P1, while the lines holding the remainder of reactants are kept still. On stopping P1 and starting P2, the sample plug is swept to the straight stretch of the tubing, namely the flow-cell (D), through which light is transmitted by means of optical fibres. As the sum of the flow-rates of the incoming (carrier and reagent) and outgoing streams is equal, the system is in hydrodynamic equilibrium.

Both variants of miniaturized FIA have a serious shortcoming, *viz.* the easy clogging of the micromanifold.

   *Gradient techniques* in FIA are based on the measurement of the analytical signal at a point other than the peak maximum. There are five basic types of FIA gradient techniques:

   (a) In the *gradient calibration* mode, a standard solution is measured to obtain a single FIA peak from which the corresponding calibration graph is run.

   (b) The *electronic dilution* mode allows the range of application of a given FIA method to be broadened by carrying out measurements at different times on the FIA peak, which is equivalent to using different sample solutions as the dispersion varies along the FIA curve [69].

   (c) *FIA titrations* can also be included among gradient techniques as they involve the measurement of the peak width at a preselected height [70- 73]. Nevertheless, they will be dealt with alongside with other types of titration in the following chapter.

   (d) The *pH-gradient* mode is based on the establishment of two opposing pH gradients upon injection of a large sample volume at a pH, $pH_1$ different from that of the carrier, $pH_2$. Strongly pH-dependent systems can therefore be used
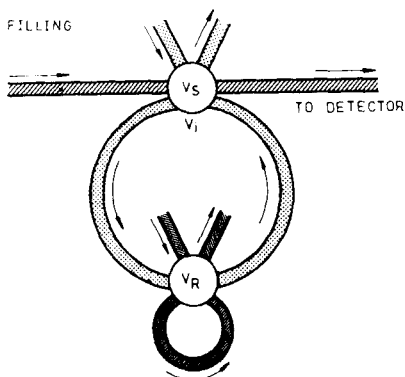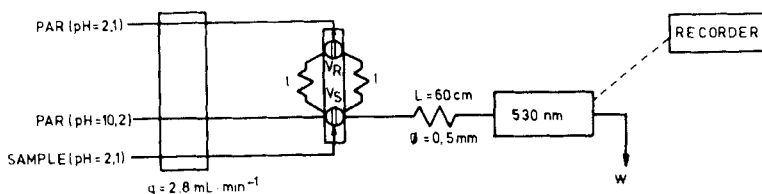
a)                                        b)
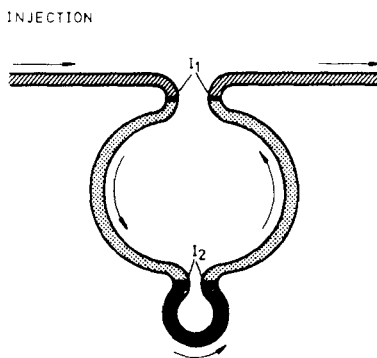


c)



**Fig. 6.12** (a) Relative situation of the two injection valves used to create a pH gradient. (a.1) Filling position ($V_s$, sample valve; $V_R$ reagent valve). (a.2) Evacuation position. A quadruple $reagent_1$-sample-$reagent_2$-sample interface is established. (b) Situation of the injection unit within the FIA system.

to carry out simultaneous determinations (e.g. that of vanadium and lead with PAR [75]). A more sophisticated manner of implementing this mode involves using a secondary injection valve (intercalated into the loop of the main valve) to insert reagent and sample at an acidic pH into a basic stream of the reagent (Fig. 6.12) [71].

(e) A *reagent concentration gradient* at the head and tail of an unusually large sample volume (2 mL) results in the appearance of two reaction zones at the ends of the plug, separated by a non-mixing zone. On passage through the detector, such zones yield two FIA peaks which can be used for kinetic determinations, either differential or of a single species (based on the signal increment between the two peaks corresponding to the time increment between the two residence times) [76].

*Kinetic methods.* FIA, on account of its intrinsic features (measurements under non-equilibrium conditions), can be considered to be a fixed-time methodology. However, according to FIA jargon, a kinetic method is based on the monitoring of the evolution of the analytical signal (stopped-flow methods) or on the measurement of two or more signals at the number of times required (differential or individual kinetic determinations).
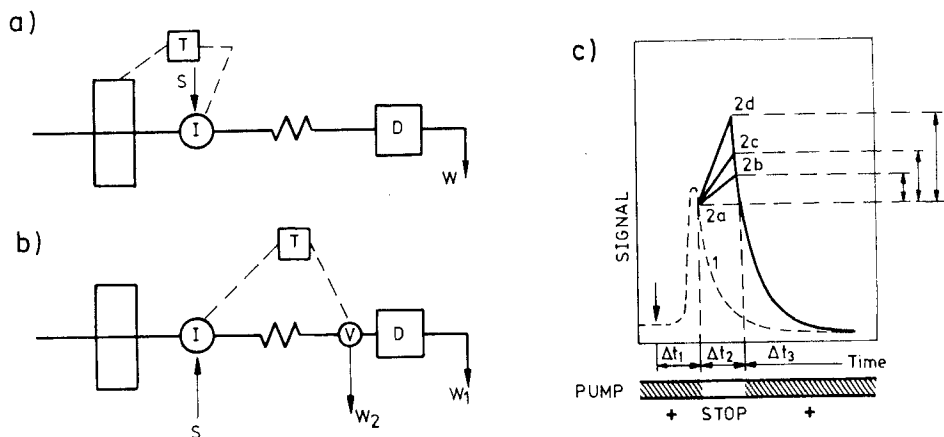


**Fig. 6.13** FIA assembly for implementation of stopped-flow methods. (a) The timer, T, synchronizes injection with the stop and start of the pumps. (b) Stopped-flow set-up featuring a three-way valve between the injection unit and the detector. The timer synchronizes injection with switching of the valve. (c) Stopped-flow recordings obtained with a dye (2a) and various systems of different reaction rates (2b, 2c and 2d).

Stopped-flow FIA methods are applicable to reactions with half-lives be-

tween 10 and 120 s. The specific additional instrumentation required to imple-
ment either of the two variants (Fig. 6.13) is simply an electronic timer syn-
chronizing injection with either the stop and start of the peristaltic pump
(Fig. 6.13a) or the switching of the diverting valve (Fig. 6.13b). In either
case, the reacting plug is stopped at the flow cell to monitor the reaction
development (Fig. 6.13c).

a)



b)



c)
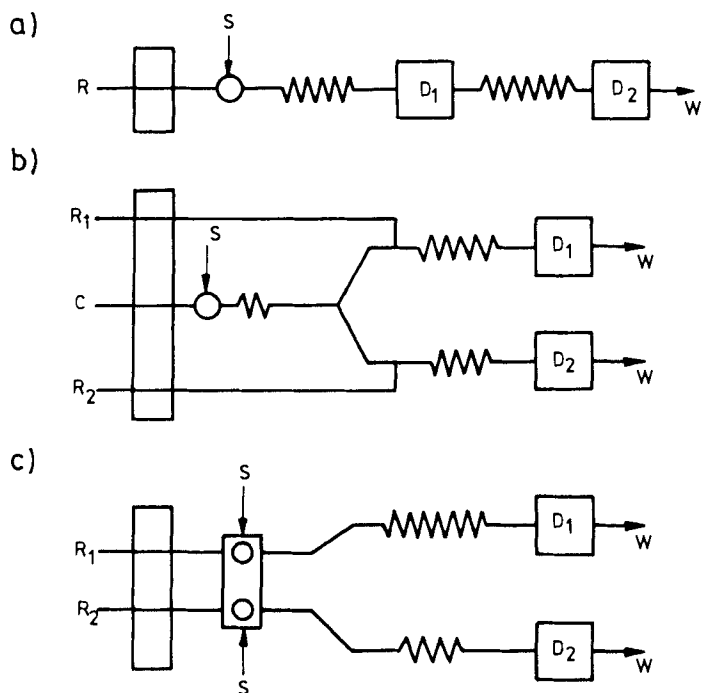


**Fig. 6.14** FIA configurations with two detectors for development of
kinetic methods. (a) Serial configuration; (b) parallel configuration
with single injection and splitting of sample; (c) parallel configu-
ration with dual injection. R, reagent; C, carrier; S, sample; D, de-
tector; W, waste.

Kinetic FIA methods, whether differential or not, can be implemented in
two ways: either by obtaining different analytical signals for a single sample
with the aid of serial or parallel detectors (Fig. 6.14), or by suitable mani-
pulation of the FIA system and use of a single detector, which undoubtedly de-
mands greater ingenuity from the experimenter. Three representative configura-
tions of this variant are depicted in Fig. 6.15. The manifold shown in Fig.
6.15a features two serial injection valves inserting the sample solution simul-
taneously into the reagent stream. The reactor located between the two valves
fixes the interval separating the passage of the two peaks through the reactor

and hence the time elapsed between the appearance of the two peaks making up
the FIA recording. The configuration depicted in Fig. 6.15b has a splitting
point and a merging point separating two channels of different geometric and
hydrodynamic characteristics intended to lead two subplugs of the injected
sample to the detector, which is reached at a different time by each of them.
The scheme in Fig. 6.15c corresponds to the use of an abrupt concentration
gradient in a large sample plug in order to obtain two reaction zones [72].
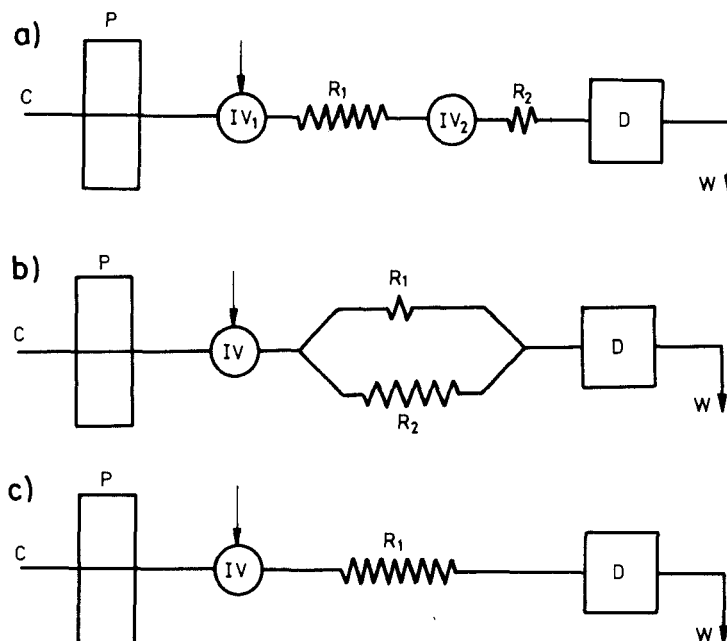


**Fig. 6.15** Types of single-detector FIA configurations used to obtain
two analytical signals at two different times from a single sample.
(a) With two simultaneously actuated serial injection valves. (b) With
splitting and merging points. (c) Single-channel system into which a
large sample volume is introduced to establish two reation interfaces.
C, carrier; P, peristaltic pump; IV, injection valve; R, reactor; D,
detector; W, waste.

Closed-open systems can also be used for individual and differential kin-
etic determinations [56,57], a relatively unexplored area in which rapid scan
detectors have great potential.

The association of FIA with separation techniques such as distillation,
dialysis, ion exchange or liquid-liquid extraction has opened up new fields of
application to the analysis of complex samples, as demonstrated in Chapter 4.

Of particular interest to FIA is the use of two-phase systems for purposes other than the separation or concentration of species, as is the case with redox columns and enzyme reactors. Redox columns used within FIA set-ups can either act upon an agent dissolved in the carrier stream which later returns to its oxidized form by reducing the unknown [77,78] or on the sample itself, in which the ingredient of interest is thus converted to an oxidation state suitable for measurement (e.g. reduction of nitrate to nitrite for individual or simultaneous determination of these analytes). Either alternative entails the use of oxygen-impervious tubing and permits determinations that are impossible by SFA. The use of enzymes as analytical reagents has grown enormously in the last few years. In FIA, these biochemical catalysts have been exploited chiefly immobilized on packed columns, open tube wall reactors, single bead string reactors and controlled pore glass reactors [79].

Liquid-liquid extraction has also been used in conjunction with the FIA technique in special cases to implement a particular determinative method rather than as a mere means of separating or concentrating certain species. One such method is the indirect determination of perchlorate by extraction as an ion pair with the Cu(I)-azine complex of 6-methylpicolinealdehyde. The amount of copper detected is a measure of the concentration of perchlorate in the sample [80].

One of the features of FIA testifying to its great versatility is its automation capability. Figure 6.16 illustrates the operation of a fully automated FIA system linked to a microprocessor via passive and active interfaces. The passive interface, fitted to the computer through the detector, collects raw data and treats them suitably to provide the analytical results matching each of the samples injected. Thus, it can perform a variety of functions such as (a) selection of the peak height, area or width data corresponding to a given signal level, or of signal increments as a function of time; (b) collection and averaging of data obtained in repetitive determinations carried out on a single sample; (c) supply of analytical data, usually obtained by transferring readings to a calibration graph and applying Lagrange's interpolation method if these fall outside the linear range, to peripherals such as a printer or plotter. The active interface allows the microprocessor to participate to a greater or lesser extent in the operation of the automated process. Hence, it is usually assigned the task of synchronizing the operation of the sampler with that of the injection unit, the valve of which should be filled and evacuated by the action of an electric motor cam. On the other hand, the microprocessor is an indispensable element of stopped-flow configurations in order to control the stop and start of the pumps (or diverting valves), as well as to fix both the delay and measurement times.
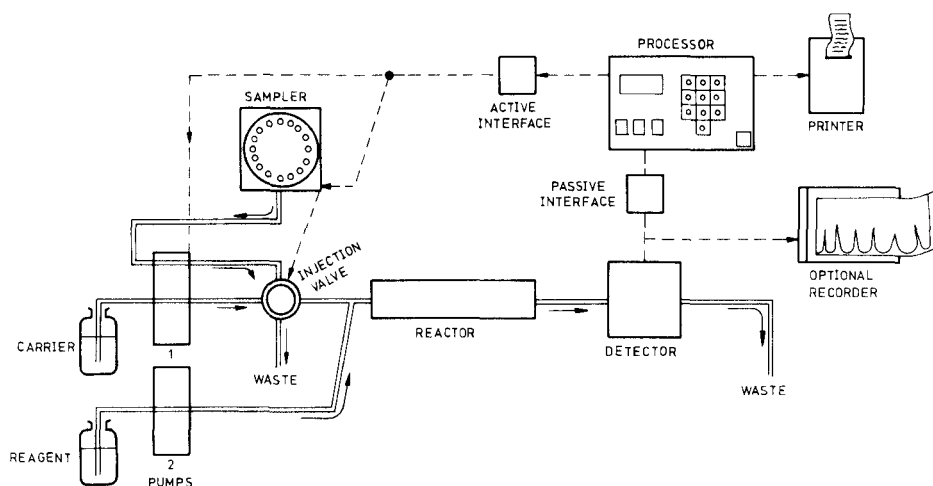
**Fig. 6.16** Scheme of a fully automated FIA system. The passive inter-
face collects data from the detector and processes them. The active
interface actuates the pumps, sampler and injection unit.

The basic set-up in Fig. 6.1 and the fully automated assembly in Fig. 6.16
represent extreme situations between which lie a variety of configurations
with different degrees of automation suited to the particular requirements
(analysis rate, number of samples, type of measurement) or chemical system in-
volved.

## 6.5 FEATURES OF FLOW-INJECTION ANALYSIS

In dealing with the most oustanding features of FIA, only those clearly
distinguishing this methodology from segmented-flow or conventional manual
methods will be considered.

### 6.5.1 Sensitivity

As a rule, FIA methods are less sensitive than manual and SFA techniques
for two basic reasons: first, the short reaction times involved result in par-
tial reaction yields; and second, the physical dispersion or dilution of the

sample in the carrier prevents the signal from attaining its maximum possible value. Little is gained in attempting to optimize the experimental conditions involved as greater reactor lengths or lower flow-rates intended to increase the reaction development result in increased dispersion and vice versa, so that compromise values must be chosen for each of the variables involved in order to obtain the best possible results. Insofar as the variables to be optimized are normally interrelated, it is usually advisable to apply the simplex method.

### 6.5.2 Selectivity

The automation or semi-automation of a conventional manual method by FIA often results in a decrease in the number and level of interferents. Thus, in the FIA version of the determination of cyanide by the classical reaction with barbituric acid/chloramine T, nitrite and sulphide pose no interference at concentrations ten times as high as that of the analyte, which is otherwise adversely affected by the presence of both interferents in the manual method [48]. The greater tolerance to foreign species in FIA methods can be generally attributed to their kinetic character, so that undesirable side reactions scarcely have the opportunity to develop to an appreciable extent in such a short interval as the residence time. The tolerance to extraneous species is even more remarkable in kinetic FIA methods based on the measurement of a reaction rate (stopped-flow). Optimization of FIA systems as regards selectivity is a relatively simple task on account of their enormous versatility.

### 6.5.3 Precision

Despite the fact that the FIA methodology involves kinetic, physical and chemical aspects which may *a priori* lead anyone into thinking that the results obtained will be anything but precise, the reproducibility levels achieved are in fact fairly high, even comparable to those attained by manual and segmented flow methods. This is possibly one of the keys to the success of FIA.

### 6.5.4 Rapidity

This is one of the most outstanding features of the FIA methodology, which clearly exceeds both manual and automatic analytical methods in this regard. The time needed to start up an FIA set-up or instrument is normally much shorter than that required to set an SFA assembly ready for measurements — usually 30 min or more. Reliable FIA measurements can generally be made within 5-10 min after starting the pump(s), as such a short time is sufficient for the flow to regularize. As a rule, the delay times, if any, in FIA are dictated by the detector warm-up.

**TABLE 6.2**

Distribution of FIA methods according to their sampling rate

| Sampling rate (samples/h) | Occurrence (%) |
|:---:|:---:|
| 0-50 | 24 |
| 50-120 | 54 |
| 120-200 | 10 |
| 200-1700 | 12 |

In any case, the rapidity of the FIA methodology is more clearly reflected in the sampling rate, which is enormously higher than that of most manual methods and two to five times higher than that of the fastest SFA techniques. Table 6.2 lists the sampling rate distribution of the FIA methods reported to date. As can be seen, 54% of the methods have sampling rates between 50 and 120 samples/h, 10% between 120 and 200 samples/h and another 24% between 0 and 50 samples/h. Nevertheless, some methods have an extremely low or high sampling rate; thus, the determination of chemical oxygen demand affords a frequency of only 10 $h^{-1}$ (the reactor length is no less than 40 m) [81], whereas the enzymatic analysis of hydrogen peroxide and glucose can be performed at an unusually high rate (1700 samples/hr) [53].

The higher sampling rate of FIA can be attributed to the occurrence of radial diffusion forces, which are much more significant than convective or axial diffusion forces, that aid in preventing carry-over by limiting the peak width through their washout effect.

### 6.5.5 Simplicity

In contrast to classical continuous flow methods, FIA is characterized by great simplicity, which in turn is the result of the combined contribution of three aspects:

(a) Simple foundation. Flow-injection methods are only a conceptual modification of SFA techniques involving replacement of the characteristic air bubbles of segmented-flow analysis with suitable geometric and hydrodynamic conditions which ultimately result in substantial advantages over classical automatic methods of analysis.

(b) Straightforward manifolds consisting of Teflon tubing, connectors, flow splitters, etc., of easy assembly, which is in clear contrast with the typical

instruments employed by the latest analytical methodologies, usually very so-
phisticated, expensive and difficult to tune up and maintain.

(c) Easy operation. FIA set-ups are not only easy to use for routine ana-
lyses, but also to adapt to the particular needs of each experimenter. Thus,
the determination of a given analyte in different matrices usually only re-
quires replacement of some component, lengthening of a reactor or a different
working temperature.

### 6.5.6 Economy

Such a straightforward technique as FIA is also naturally inexpensive, in
clear contrast to other automatic methodologies, both continuous segmented and
batch. On average, an FIA instrument is two to five times cheaper than an auto-
matic instrument featuring similar or even poorer performance. In this regard,
it is interesting to note the possibility of assembling inexpensive home-made
configurations from commercially available parts. Such assemblies are much
cheaper than commercial instruments, which has probably been the chief reason
for the meagre commercial development of this novel technique.

Flow-injection analysis is also an economical technique as regards reac-
tant consumption, which is usually 2-15 times lower than in SFA and 20-100
times lower than in conventional manual methods.

### 6.5.7 Versatility

The modular character of an FIA system allows it to be readily adapted to
the particular needs of each type of analysis. Component replacement is a
relatively easy task as high pressures are not used. On the other hand, the
optimization of a purpose-built FIA system poses few problems if the right
alternative is applied (e.g. the simplex method).

The flexibility of this methodology allows for a variety of applications:
from determinations with no additional chemical reaction to sequential reac-
tions involving five or six reactants; from gradient techniques to stopped-
flow or kinetic determinations; from incorporation of liquid-liquid extraction
units to insertion of gas samples.

Flow-injection analysis is also an excellent substitute for manual methods
involving classical problems. Thus, the possibility of incorporating reducing
columns into the system allows the use of strong reductants (e.g. $Cr^{2+}$ or $V^{2+}$)
with no special precautions. The ease with which typical analytical
instruments such as photometers and pH-meters can be incorporated into FIA
assemblies further adds to its versatility.

### 6.6 APPLICATIONS OF FIA

The broad scope of application of FIA is a consequence of its flexibility,

which is responsible for its widespread use in clinical [82], pharmaceutical [83], nutritional [84] and environmental [85] chemistry. The most significant contributions of FIA to these areas are described in detail in Chapters 13-15.

Flow-injection analysis, as stated earlier in this chapter, offers a host of substantial advantages over conventional analytical methodologies, namely automation capability, flexibility, low reactant consumption, great versatility, etc. Thanks to its automation capability, the FIA technique is a major alternative to many continuous flow methods in areas such as clinical chemistry which require the processing of a large number of samples in a time as short as possible.

FIA is compatible with standard-addition methods [86,87] and with other calibration techniques such as electronic calibration [70] and exponential dilution [7]. The gradient dilution technique, developed by Tyson *et al.* for application in atomic absorption spectrometry, features further advantages such as rapidity, low sample and reagent consumption and no need for volumetric glassware. In this context it is worth mentioning a procedure for injection of standard solutions into the sample stream in order to effect calibrations by the standard-addition method, as well as a convenient method for the determination of the tolerance level afforded       [86,88]. Another interesting contribution in this field is represented by the creation of exponential concentration gradients with the aid of a gradient chamber [86-88]. A classical mathematical equation permits an unknown concentration to be calculated by measuring the time needed for a given absorbance value to be attained.

Several mathematical expressions describing the variation with time of the concentration of a sample in a large volume first injected into a flowing stream and then passed through a mixing chamber have been reported by different workers. Tyson *et al.* [86] derived the following simplified experimental relationship describing the profile of the evolution of the sample-carrier interface:

$$C = C_0[1 - \exp(-qt/V_g)] \tag{6.10}$$

The plot of C against t is a typical rising exponential curve starting at C ❑ 0 (for t = 0) and tending to $C_0$ at sufficiently long times, which obviously depend on the flow-rate and the gradient chamber volume. If it is the carrier that is injected into the sample stream (i.e. some dilution is involved), the expression above becomes

$$C = C_0 \exp(-qt/V_g) \tag{6.11}$$

This is representative of a falling exponential curve. Pungor *et al.* [89] found analogous expressions in developing a procedure for the calibration of ion-selective electrodes.

Figure 6.17 shows the experimental set-up proposed by Tyson *et al.* [86] for gradient calibrations. It consists of two reservoirs holding water and a concentrated standard solution of the metal ion, linked via a three-way valve which allows the two solutions to be sent separately to the nebulizer.

Water

Concentrated solution [$M^{n+}$]

Atomic absorption

Three-way rotary valve

Gradient chamber

Recorder
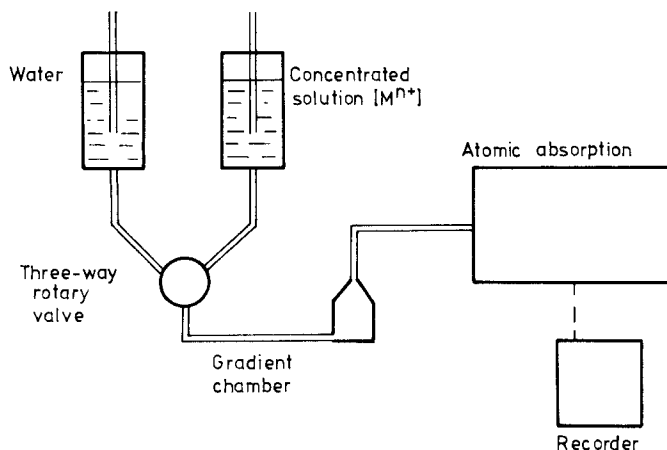
**Fig. 6.17** Calibration system for AAS with gradient chamber.

When low sample and/or reagent consumption is required because of the inaccessibility (biological fluids), expense (enzymes) or detriment caused to the sensing system (e.g. highly saline samples intended for analyses by atomic absorption spectrometers [44]), FIA modes such as merging zones [60–63] or integrated microconduits [67,68] offer considerable advantages over other methodologies.

Flow-injection analysis is suitable for use with chemical systems with very different reaction rates. Thus, fast reactions are usually treated by normal FIA, whereas slow processes are generally dealt with by other modes such as zone trapping [64] or stopped-flow techniques [61].

Unstable chemical systems, which cannot be investigated by many other techniques, can be conveniently studied by FIA. Thus, determinations involving reagents liable to be affected by contact with the atmosphere (e.g. strong reductants [77,78]) or systems yielding unstable monitored products [60] pose hardly any problem when carried out by FIA, which is also an invaluable tool for the determination of acid–base constants of easily hydrolysable substances [90].

When the analyte concentration falls outside the analytical range of the method in question, FIA allows the implementation of amplification and dilution techniques with the aid of:

(a) A microprocessor [70],

(b) a closed–open single-detector multi-detection system allowing recordings such as that shown in Fig. 6.18 to be obtained, from which the beam of straight lines in Fig. 6.18b is obtained, either on the basis of the sum of the absorbances of different peaks (amplification method) or by use of a single peak other than that of maximum absorbance (dilution method) [56]; or

(c) a multi-detector (e.g. an on-line diode spectrophotometer) either for addition of the absorbance values at the maximum and in a neighbouring zone on both sides of the maximum or for taking a single measurement wavelength more or less distant from the absorption maximum of the chemical system (dilution method) [91].
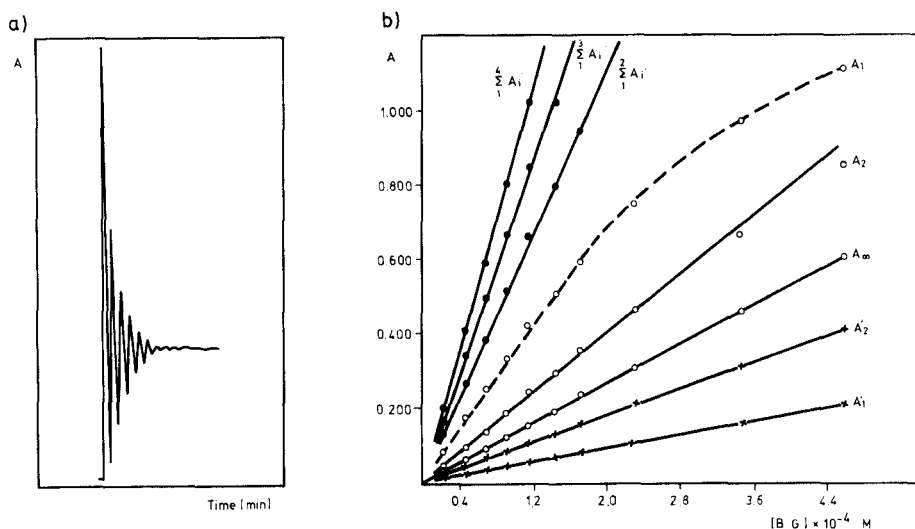


**Fig. 6.18** (a) Typical recording obtained by injecting a dye (Bromocresol Green, BG) into a closed-open system. (b) Straight line beam obtained by applying the amplification and dilution technique to BG. $A_1$, straight line obtained from the first peak (dotted line), as usual in FIA. $A_2$, $A_\infty$, $A'_2$ and $A'_1$ correspond to the measurement made at the maximum of the second peak, absorbance at equilibrium, minimum of the second peak and maximum of the first peak, respectively. $\sum_1^4 A_i$, $\sum_1^3 A_i$ and $\sum_1^2 A_i$ denote the sum of the absorbances of the first four, three and two peaks, respectively.

The development of kinetic methods (both stopped-flow and differential) by FIA offers substantial advantages over conventional methodologies.

The possibility of carrying out simultaneous determinations of several analytes in a single sample by FIA has hardly been exploited so far, although it is a highly promising aspect on account of its great versatility. Such a possibility is of special relevance to two areas of analysis, namely clinical and environmental chemistry. A summary of the most common configurations used for simultaneous determinations carried out with a single detector is presented in Fig. 6.18. The manifold in Fig. 6.18a, described above, has allowed the development of differential kinetic methods for the analysis of Ni-Co [93], pyridoxal/pyridoxal-5-phosphate [94] and phosphate/silicate mixtures, while manifold b can be utilized for both non-kinetic and differential kinetic determinations by making $R_1 = R_2$ (e.g. determination of phosphate and silicate [95]). The reversed FIA manifold depicted in Fig. 6.19c has been employed for the simultaneous non-kinetic determination of water pollutants
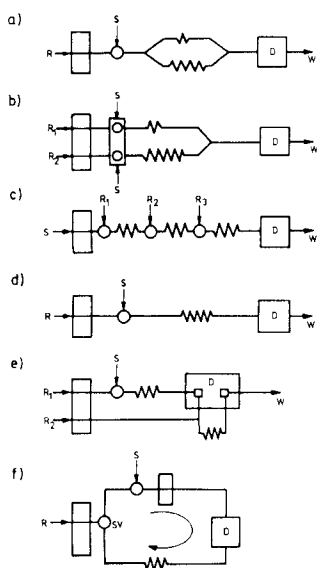


**Fig. 6.19** Manifolds used for simultaneous determinations with a single detector. (a) With splitting and merging points; (b) with dual injection and one merging point; (c) reversed FIA with sequential injection of reactants; (d) injection of a large sample volume and formation of two reaction zones; (e) manifold with two serial flow-cells accommodated in a spectrophotometer; (f) closed-open system. R, reagent; S, sample; D, detector; W, waste; SV, selecting valve.

[49,51], whereas the configuration shown in Fig. 6.19d has been applied to simultaneous non-kinetic determinations based on pH-gradients (e.g. Pb-V [74] and Co-Cu [96]) and to the differential kinetic determination of Ni and Co [76] by use of a reagent concentration gradient. Manifold *e* in Fig. 6.19 is useful for both types of determination: differential kinetic (e.g. Ni-Co [97]) and non-kinetic (chromium speciation [98]). Finally, multidetection has been used in conjunction with open-closed systems for the differential kinetic determination of Cu and Fe [91], in addition to other applications commented on above.

The configurations usually employed for simultaneous determinations by use of two detectors (Fig. 6.14) were also dealt with above.

It has been plausibly demonstrated that the development of a method using FIA leads to a substantial decrease in interferences [52] in relation to the manual alternative. This is probably a result of the kinetic nature of flow-injection measurements. On the other hand, it has been irrefutably proved that the on-line incorporation of continuous separation devices such as liquid-liquid extractors, ion exchangers, dialysers or distillation units is of con-
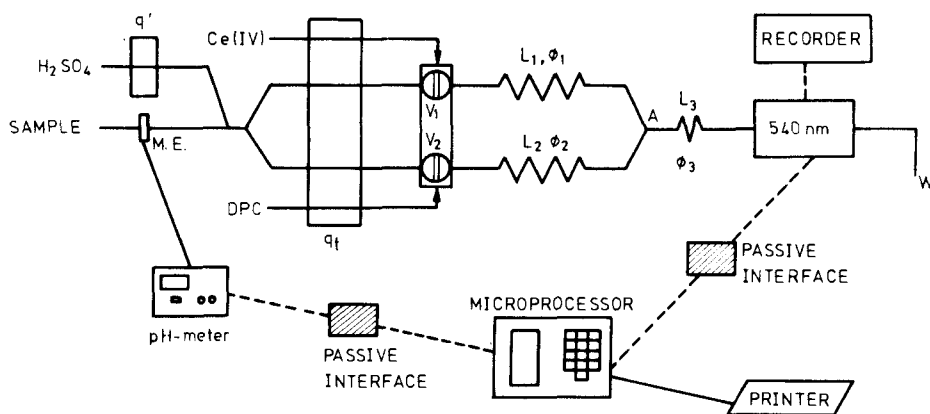


**Fig. 6.20** FIA configuration for the speciation of chromium. ME, glass-calomel microelectrode; DPC, 1,5-diphenylcarbazide; $q_t$, (total flow-rate) = 3.26 mL/min; q' $\square$ 0.30 mL/min; $V_1$ and $V_2$, injection valves (1300 and 475 µL); $L_1$ = 650 cm; $L_2$ = 360 cm; $L_3$ = 60 cm; $\phi_1$ = $\phi_2$ = $\phi_3$ = 0.5 mm.

siderable use for preconcentration operations and/or interference removal. Flow-injection analysis is suitable for the determination of physico-chemical parameters such as acid-base [90] and complex-formation constants [99], refractive indices [100] and viscosities [101].

In its reversed mode, FIA is fully adaptable to industrial process control [49-51], and to studies of the speciation of various elements in waters [102]. One of the most promising developments in the latter area is the speciation of chromium by means of the configuration depicted in Fig. 6.20. This uses a combined glass-calomel microelectrode incorprorated in the sample stream prior to the simultaneous injection of the reagents (Ce$^{4+}$ and 1,5-diphenylcarbazide for Cr$^{3+}$ and Cr(VI). The data obtained for the concentration of these two species, together with the sample pH and the constants corresponding to the equilibria in which both oxidation states are involved, allow the calculation of the concentration of up to nine different chromium species: aquo complexes and hydroxylated forms of Cr(III) and ionic, molecular and dimeric forms of Cr(VI) [103].

These are but a few aspects in which the FIA methodology competes advantageously with or even excels other automatic flow analysis methods.


## 6.7 TRENDS IN FIA

Flow-injection analysis is currently at a stage of almost exponential growth judging by the large number of papers devoted to unravelling its principles and broadening its scope of application every year. The significance of this novel methodology and its impact on the  scientific community can be assessed from its treatment as a separate discipline at international meetings, as well as from its virtually constant presence in the latest issues of the leading analytical journals. The discussion below advances likely future trends in FIA regarding its foundation, instrumentation and applications.

As regards the foundation of FIA it is reasonable to assume that the near future holds the final establishment of the theory allowing the geometric and hydrodynamic characteristics of an FIA assembly to be accurately related to the parameters defining the FIA recording. This will undoubtedly call for the development or computer-simulated FIA processes and experimental comparisons It is also probable, and certainly desirable, that accurate relationships between chemical kinetics and the physical processes involved will be finally derived.

As far as instrumentation is concerned, manufacturers are expected to supply dedicated instruments suited to present needs (e.g. multi-channel apparatuses for simultaneous determinations). More accurate and readily controlled

propelling systems, the use of fibre optics and further miniaturization are also to be expected. The growing affordability of microcomputers will almost certainly make them indispensable components of every FIA set-up.

In the context of FIA applications it is natural to predict that clinical chemistry and environmental pollution will be the two most appealing fields to this methodology. Flow injection analysis is also bound to bring significant changes in the future orientation of kinetic methods of analysis.

It should be noted that FIA is not exclusive to analytical chemistry. Thus, Betteridge has claimed that the new methodology is indeed a major alternative for the acquisition of comprehensive chemical information in a simple, fast and inexpensive manner [104].

As stated by prestigious researchers, the only limitation of FIA is the user's imagination [104-106].

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# 7
## Automatic continuous analysers. III.
## Other automatic unsegmented flow methods

## 7.1 INTRODUCTION

This chapter deals with a variety of automatic methods with very peculiar features that distinguish them from those described in the preceding chapters and make them applicable to particular problems in areas such as completely continuous flow analysis (e.g. waste water analysis) and clinical analysis. These methods can be classified into three general groups, namely *continuous mixing methods*, *stopped-flow continuous mixing methods* and *continuous-flow titrations*.

### 7.1.1 Continuous mixing methods

These involve introducing the sample into the system, mixing it with the carrier or reagent, measuring the reacting plug as it passes through a suitable detector and either sending it to waste (open systems) or recirculating it in order that the analyte may partition between the two phases upon contact with the immiscible phase (closed systems). Alternatively, the sample can be introduced into the system in an intermittent fashion and wash cycles can be intercalated between samples to avoid carryover. In some instances, the determination consists of two well-defined stages: (a) deposition of the analyte with removal of sample and (b) aspiration of buffer prior to detection (e.g. in the determination of heavy metals in waters as proposed by Cnobloch and based on the preconcentration-redissolution principle).

### 7.1.2 Stopped-flow continuous mixing methods

The flow can be stopped at various stages during the process. In the variant of sample insertion without injection, the purpose of stopping the flow is to prevent air from entering the system between sample aspiration and reagent aspiration or washing. In methods based on fast kinetics, the flow is halted at the detector in order to monitor the evolution of the reaction, whereas the idea behind stopping the flow in SF/USA is to refill the syringe after each injection.

### 7.1.3 Continuous-flow titrations

In these, the sample is introduced into the system in a continuous manner, either by keeping its speed constant and changing that of the titrant, or vice versa —alternatively, both sample and titrant can be kept at a constant speed and measurements can be made as a function of the analytical signal thus obtained. A discontinuity is occasionally introduced into the system in order to intercalate the wash solution; other times (e.g. in FIA titrations, which involve injecting the sample into the titrant stream and monitoring the signal/time binomial) there is no need to stop the flow as the titrant stream itself acts as a wash solution.

**TABLE 7.1**

Types of discontinuity in automatic methods

| Type of method | Features of the discontinuity |
|---|---|
| Batch, robotic | No flowing stream. Samples held in separate cuvettes |
| Continuous | |
| – Segmented | Air bubbles/wash solution |
| – Unsegmented | |
| (a) Sample (reagent) injection | |
| a.1 Into a continuous carrier or reagent stream | Single serial injection system |
| a.2 Simultaneous mixing of sample and reagent (stopped-flow) | Dual parallel injection and halting of flow |
| (b) Sample aspiration | |
| b.1 Without halting of flow (CCFA) | No discontinuity |
| b.2 Intermittent introduction | |
| . Without halting of flow | Sample changeover |
| . With halting of flow (CDFA) | Intermittent pumping/sample changeover |

None of the methods considered here strictly uses segmentation by air bub-

bles. Whenever air is introduced, its function is different from that in SFA (e.g the determination of total mercury by CCFA, in which the air present facilitates the diffusion of mercury vapour).

An overview of the different types of discontinuity used in automatic methods and their characteristics is presented in Table 7.1. The most common discontinuity in discrete and robotic methods is the absence of flow, which involves keeping the samples in separate vessels for measurement. On the other hand, automatic continuous methods use very different kinds of discontinuity or do not use one at all. The discrete nature of segmented methods is determined by the presence of bubbles and wash cycles as a means of avoiding carry-over, whereas that of unsegmented methods is dictated by the manner in which the sample —and reagent— is introduced into the system. There is only a single type of method using no discontinuity: *completely continuous flow analysis* (CCFA).

## 7.2 CONTINUOUS MIXING METHODS

Under this heading are distinguished two groups of methods according to the way in which the sample is introduced; this can be either incorporated into the system (open or closed) by insertion into the carrier in a continuous fashion or introduced intermittently into it, with intermediate wash cycles between successively injected samples in order to avoid carry-over.

### 7.2.1 Continuous introduction of sample

Automatic continuous-flow methods involving the continuous introduction of sample into the system are implemented by means of two different configurations: (a) *open*, in which the flow is wasted after passing through the measuring cell, and (b) *closed*, in which the flowing solution is returned to the vessel to be recirculated once it has passed through the detector.

#### *7.2.1.1 Open systems*

Open configurations, the general scheme of which is depicted in Fig. 7.1, involve evolving systems. The extent to which the system concerned has evolved is continuously monitored through the changes detected by the sensing system on mixing the sample with a suitable reagent which is discarded after measurement. The outstanding feature of this type of system is the use of a single sample, the evolution of which is monitored as a function of time. As only one sample is employed, no wash cycle is required; it suffices to follow the changes occurring in the concentration of the analytes. These systems are thus ideally suited to the monitoring of water, industrial effluents, etc. —abun-
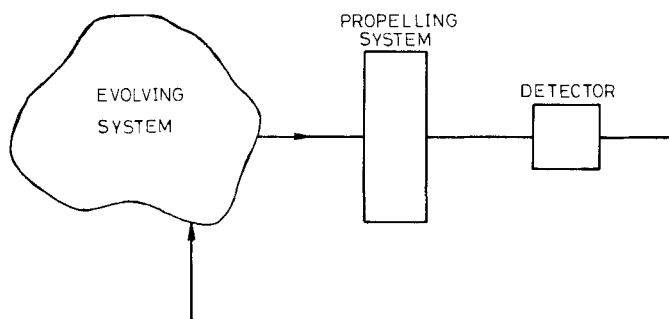
dant and inexpensive samples requiring the continuous control of various species.

a)
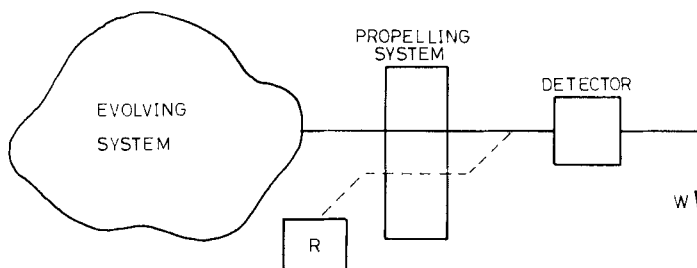


b)



**Fig. 7.1** Basic types of configuration used in completely continuous flow analysis. (a) Open: the sample is discarded after measurement. (b) Closed: the sample is recycled.

This methodology was first implemented in the completely continuous flow analyser (CCFA) developed by Goto [1] for the continuous monitoring of chemical oxygen demand (COD) in waste water and of total mercury in water. He determined COD by use of a configuration such as that depicted in Fig. 7.2a, in which the sample stream is mixed continuously and successively with an acidic solution of 40% $H_3PO_4$ and 10% $H_2SO_4$, a 5% solution of $AgNO_3$ and a 16mM solution of $KMnO_4$. The slow kinetics of the redox reaction involved calls for the use of an 18-m reactor immersed in a thermostated bath at 100°C. The stream finally reaches a thin-layer electrolytic flow-cell working at a constant potential of 0.50 V against an Ag–AgCl electrode. Figure 7.2b shows the recording obtained by amperometric monitoring of permanganate reduction in laboratory waste throughout an afternoon (from 4.00 to 8.30 p.m.). Note the low flow-rates used (100 and 50 µL/min for the sample and reagent, respectively), which result in decreased reagent consumption.

a)
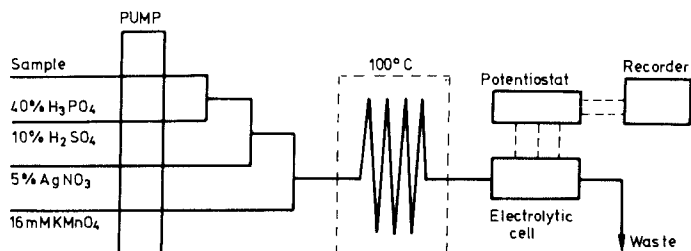


b)
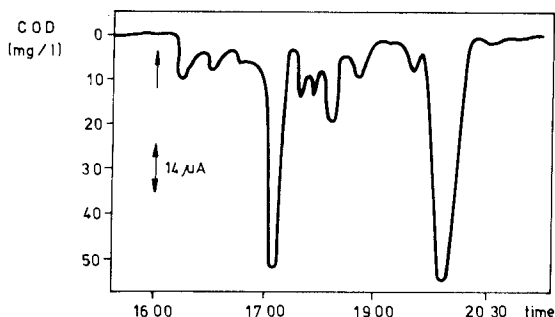


**Fig. 7.2** (a) Scheme of a completely continuous flow analyser for un-
interrupted determination of chemical oxygen demand. (b) Results ob-
tained in the monitoring of laboratory waste throughout an afternoon.
(Reproduced from [1] with permission of the Royal Society of
Chemistry).

The continuous monitoring of total Hg was accomplished by Goto [1] with
the aid of the configuration displayed in Fig. 7.3a, in which the sample is
aspirated at a relatively high flow-rate (3 mL/min) through A and mixed contin-
uously with an acid stream, D (50% $H_2SO_4$) and an oxidant, E (4% potassium per-
sulphate). It is then introduced into a Teflon reactor (1.0 mm bore, 10-m
long) immersed in a thermostated bath at 80°C, where the organic compounds
present in the sample are oxidized and organic mercury is converted into inor-
ganic metal. The digested sample is mixed with the reductant, B (10% $SnCl_2$ in
10% HCl) and air (C), subsequently being driven to the reductionextraction
reactor (polyethylene tubing), where Hg(II) is reduced to elemental mercury
which diffuses to the gas phase. The sample is later led to a gas-liquid sep-
arator in which the liquid is sent to waste while the vapour is carried by the
air stream through the condenser —immersed in a water-bath—, where any steam
present in the gas phase is condensed and passed to waste. The dry mercury
vapour passes through the flow-cell of a UV spectrophotometer, which monitors
the absorbance at 353.7 nm. Figure 7.3b illustrates the results obtained on
monitoring laboratory waste for an afternoon. The determination, like that of

COD, is carried out at a low reagent flow-rate (0.1 mL/min), although that used for air is higher (3.0 mL/min).

a)



b)
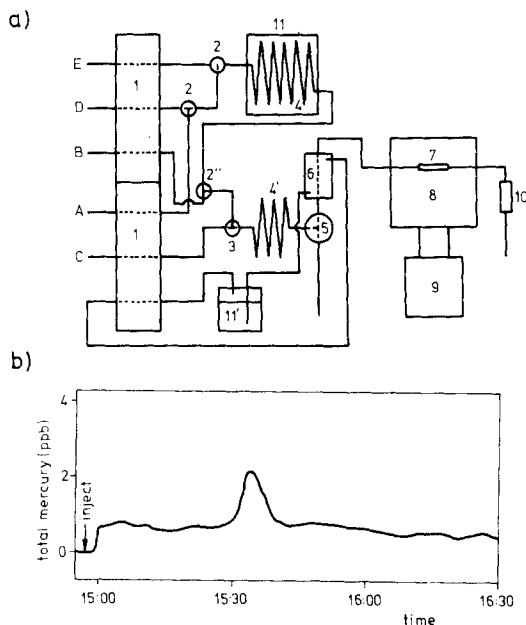


**Fig. 7.3** Diagram of analyser for continuous monitoring of total mercury in water: 1 and 1', peristaltic pumps; 2, 2', 2" and 3, merging points; 4, reaction-oxidation tube; 4', reduction-extraction tube; 5, gas-liquid separator; 6, condenser; 7, UV flow-cell; 8, UV spectrophotometer; 9, recorder; 10, mercury vapour absorber; 11, water-bath (0°C); A-E, sample, reductant, reagent, acid and oxidant stream, respectively. (Reproduced from [1] with permission of the Royal Society of Chemistry).

The monitoring of such a highly toxic anion as cyanide has also been carried out by this completely continuous methodology [2] by use of a classical spectrophotometric system (barbituric acid/chloramine T) and the configuration shown in Fig. 7.4a, in which a stream of chloramine T is mixed with the sample and then merged with a pyridine/barbituric acid stream after the corresponding reactor. A second reactor allows the coloured product to form and be monitored at 578 nm. This method, also implemented by normal and reversed FIA, allows a comparison between these two modes and CCFA. In addition, it delimits the scope of application of each configuration: continuous monitoring of abundant and inexpensive samples for completely continuous flow analysis or reversed FIA —if only sporadic control is required in the latter case— or of expensive or scanty samples for normal FIA.
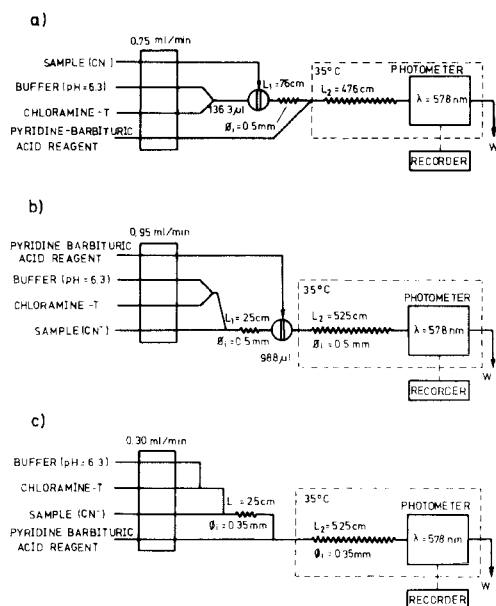
**Fig. 7.4** Configurations constructed for the determination of cyanide in water. (a) Normal FIA method (periodic analyses of valuable or scanty samples). (b) Reversed FIA method (periodic analyses of abundant, inexpensive samples). (c) Completely continuous method (uninterrupted analysis in evolving systems). (Reproduced from [2] with permission of Pergamon Press).

### 7.2.1.2 Closed systems

This type of configuration is shown schematically in Fig. 7.1b. Its chief use is with two-phase systems in which one of the phases, after passage through a suitable separator, is continuously pumped to the sensing system, which monitors the changes in the chemical system. The applications described so far are connected with studies of extraction kinetics or titrations.

Figure 7.5 shows the configuration designed by Freiser *et al.* for the study of extraction kinetics [3]. It represents an improvement over a non-automatic instrument previously devised by Freiser's group [4]. The configuration concerned, which was applied to the extraction of Ni(II) with dithizone, consists of a reservoir (immersed in a thermostated bath) into which the sample is introduced through inlet C. The reservoir is furnished with a high-speed stirrer, a Teflon phase separator capable of selectively filtering water-immiscible solvents from an intimate admixture with the aqueous phase and situated at the end of a piece of tubing leading to the flow-cell of a spectrophometer.

Once the organic phase has been separated, it is introduced into the flow-cell, G (volume 50 µL), fitted to the peristaltic pump, I. The spectrophotometer is connected to a recorder (J), an analogue-to-digital converter (K) and a clock (L), and interfaced to a microcomputer (M), a floppy-disk drive (N) and a printer or digital plotter (P). The use of suitable software allows correction for baseline drift and anomalously large absorbance readings arising from possible passage of air bubbles through the flow-cell. The calculation of the rate constant of extraction of Ni(II) by dithizone in the organic phase is based on the use of hundreds of experimental points and is made over at least two half-lives in order to allow its utilization over a wide range of reaction rates. As absorbance readings are made on the organic phase, the reaction development can be followed by measuring either the decrease in the free extractant concentration or the increase in the chelate concentration in this phase.
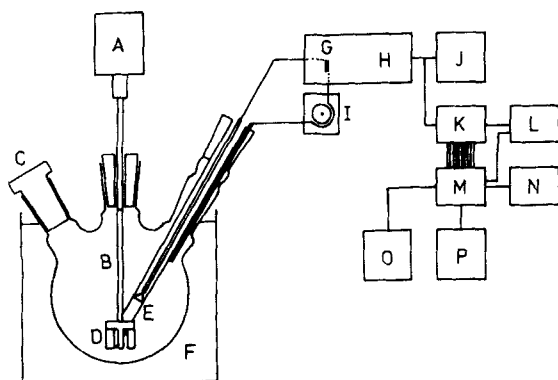


**Fig. 7.5** Instrumental design for automatic study of extraction kinetics. (Reproduced from [3] with permission of the American Chemical Society).
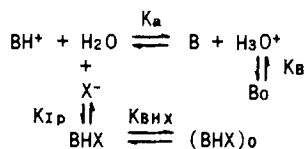
The instrument illustrated in Fig. 7.6a, similar to that described above, has been applied to the photometric titration of weakly acid drugs in the presence of an immiscible solvent. The essential differences between this instrument and the previous one lie in the use of a spoiler aimed at minimizing vortex formation arising from the utilization of a stirring bar; a burette dispensing the titrant or the washing solution and a triple layer of filter paper on the Teflon membrane to allow it to be traversed by the aqueous phase which, in turn, is propelled to the flow-cell by means of a peristaltic pump [5].

a)



b)
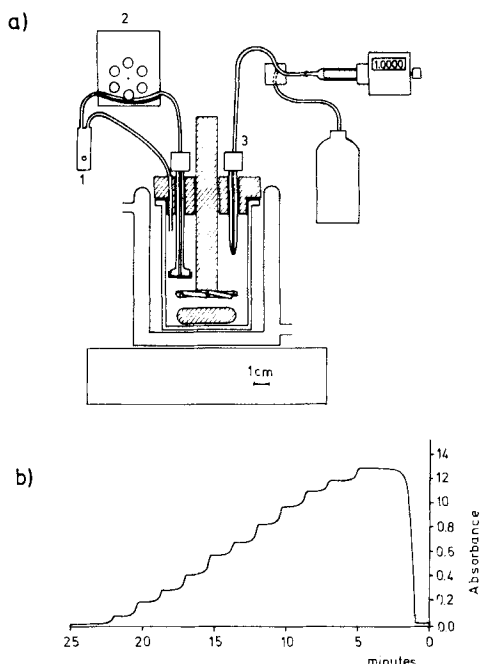


**Fig. 7.6** (a) Automated  instrument for acid–base titration  of drugs.
1, Flow-cell; 2, peristaltic pump; 3, automatic burette. (b) Recording
used to  construct the  calibration graph.  (Reproduced from  [5] with
permission of the American Chemical Society).

The procedure followed in the determination is as follows: 10 mL of the
aqueous solution of sample are placed into the titration vessel together with
20 mL of $CHCl_3/CCl_4$ (1:1) and the system is then started. After 4 min is the
following equilibrium is attained

$$
\begin{array}{ccc}
 & K_a & \\
BH^+ + H_2O & \rightleftharpoons & B + H_3O^+ \\
+ & & \updownarrow\ K_B \\
X^- & & B_0 \\
K_{Ip}\ \updownarrow\quad K_{BHX} & & \\
BHX & \rightleftharpoons & (BHX)_0
\end{array}
$$

whence the absorbance of the aqueous solution reaching the flow-cell stabi-
lizes. At this point the titrant is added at 1.5-min intervals so that the
reaction

$$
\begin{array}{cc}
 & OH^- \\
B + H_3O^+ & \longrightarrow\quad 2H_2O \\
\updownarrow & K_H \\
(B)_0 &
\end{array}
$$
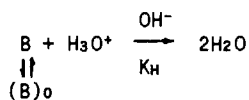
may be completed and the aborbance may reach a new plateau (Fig. 7.6b). The titration curve is traced by plotting the values of the different absorbance plateaux as a function of the volume of titrant added (mL), allowing for the dilution effect. The time needed to attain stability after each addition of titrant is dependent on (a) the stirring efficiency, (b) the pumping rate, (c) the fraction of the aqueous phase contained in the pumping system and flow-cell and (d) the flow pattern through these two elements.

This configuration allows for replacement of the hydrophilic filter paper by silicone-treated hydrophobic paper, which is permeable only to the organic phase and hence permits inversion of the monitored phase. The instrument can also be utilized for complexometric titration of metal ions by use of ligands that form extractable complexes with the ions.

### 7.2.2 Intermittent introduction of sample

This mode of sample introduction is used when dealing with distinct samples whose successive analysis requires two major demands to be met:

(a) Periodic changeovers of the sample solution, which involve stopping the aspiration in one of the reservoirs and starting it in another. Inasmuch as the propelling system operates in a continuous fashion, sample changeover includes an interval during which the end of the aspiration tube is out of contact with the solution so that some air enters the system.

(b) Wash cycles to be intercalated between successively inserted samples. Obviously, the more different the samples are the longer is the wash period required, and the lower the analysis rate.
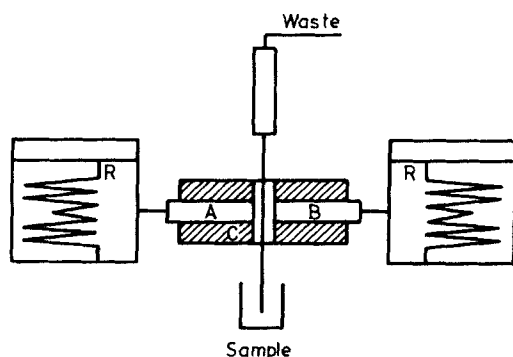


**Fig. 7.7** Simultaneous analyser for glucose and urea: A and B, enzyme electrodes for these species; R, recorders; C, flow-cell (40 μL).

The inherent features of these methods make them particularly suitable for clinical analyses, with either electrical or optical detection of the analytical signal, which is measured as such (non-kinetic methods) or monitored as a function of time (kinetic methods). Some representative examples are commented on below.
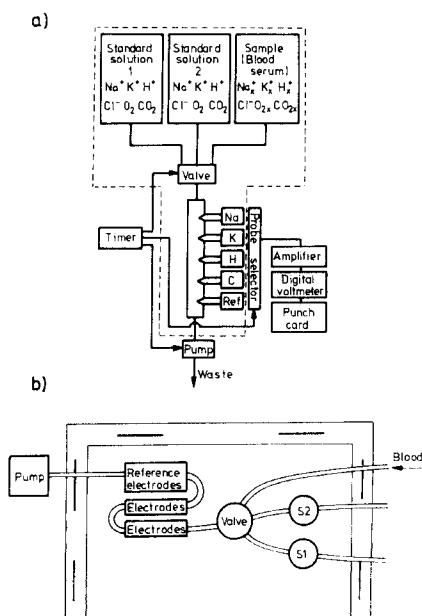


**Fig. 7.8** (a) Diagram of a multiple analyser for determination of $Na^+$, $K^+$, $H^+$ and $Cl^-$ by means of serial potentiometric detectors. (b) Enlarged section of the valve used for selection of sample and standards. (Reproduced from [7] with permission of the American Chemical Society).

Mascini and Paleschi [6] proposed the straightforward set-up shown in Fig. 7.7 for the simultaneous determination of glucose and urea in serum samples by use of home-made electrodes incorporating enzymes immobilized on nylon nets fixed on oxygen or ammonia sensors. The electrodes are placed facing each other within a single flow-cell (40 µL) through which the sample is aspirated for 1 min at a flow-rate of 1.8 mL/min), followed by a wash period of 2 min with a buffer intended to restore the baseline between successively injected samples. The recorder connected to each sensor allows the collection of the signals corresponding to each sample. In Fig. 7.8a is shown a serial configuration of sensors used for the determination of up to four inorganic species

[7]. It permits the continuous sequential pumping of the sample plus two calibration standards, as well as acquisition and treatment of the data corresponding to the concentration of $Na^+$, $K^+$, $H^+$ and $Cl^-$ in blood serum. The dotted line in the figure represents the thermostating system used (38°C). A rotary valve (Fig. 7.8b) driven by a low-speed motor determines the order in which each of the three streams is sent to the sensing system.

Amperometric detection has been utilized in conjunction with this type of method for the determination of both lactate by use of an oxygen sensor covered with a nylon membrane supporting immobilized enzyme [8] and penicillins [9] with differential amperometric monitoring and splitting of the sample into two substreams (Fig. 7.9), one of which acts as a blank and the other causes the analytical reaction involving oxidation of penicillin to penicilloic acid (which is determined iodometrically) by means of penicillinase or an alkali base. Hence, the only difference in composition between the solution circulating through the reaction manifold and the blank is the absence of an oxidant from the latter. The two tubular Pt electrodes used, one per line, are bridged to a common reference electrode (SCE). The peristaltic pump supplies a pulsating flow which undoubtedly influences the appearance of the recordings. To circumvent this shortcoming, the sample is pumped to waste at a lower rate than that at which it is aspirated into the system and excess liquid is evacuated through $PS_1$ and $PS_2$.
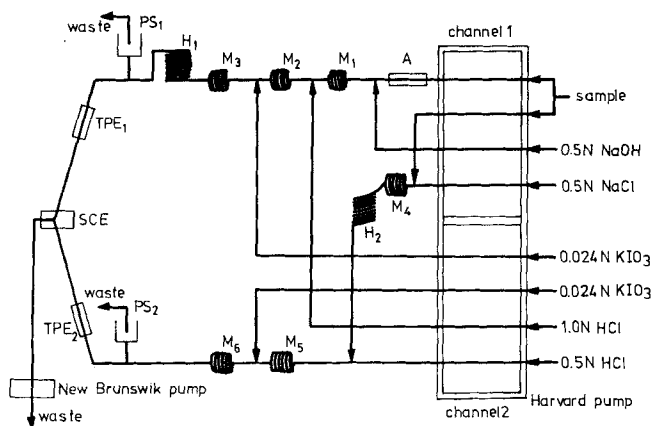
**Fig. 7.9** Scheme of instrument for enzymatic determination of lactate by differential amperometric measurements. The chief difference between the measurement (top) and reference manifold (bottom) is the absence of an oxidant from the latter. (Reproduced from [8] with permission of Elsevier).

A relatively simple configuration that can be included in this section was

developed by Cnobloch et al. [10] for the determination of heavy metal ions in natural and waste waters on the basis of the electrolytic preconcentration principle and coulometric detection. The set-up used is described in detail in Chapter 14. The method applied involves deposition of the metals concerned on the cathode (which is maintained at a constant potential and where stripping and measurement of the electric charge involved in the process are carried out), evacuation of the cell and subsequent loading with pure electrolyte.

The interesting system proposed by Weisz and Fritz [11] uses both electrochemical and optical techniques for a number of catalytic determinations, all of which are based on controlled anodic generation of a catalyst and spectrophotometric monitoring of the catalysed reaction. In this manner, with the aid of the instrument depicted in Fig. 7.10, they determined Fe(III). The reagents utilized are propelled into the mixing chamber (MC), where they mix with the catalyst generated at G, which in turn is swept by the $Na_2SO_4$ solution. The reacting mixture enters the vertical cuvette C, which is traversed by a light beam previously selected by a suitable filter. After passing through the solution held in C, the beam reaches the photoelement (PE). The absorption signal obtained is plotted as a function of time and is a measure of the analyte concentration and hence of the reaction rate. The solution emerging from the cell is led to a levelling bottle, the height of which is fixed so that the solutions pass through the cuvettte as quickly as they enter the mixing chamber. If all the reactant concentrations are kept constant, the signal provided by the photoelement is a measure of the concentration of catalyst produced in the generation cell. Catalysts such as Cu(II) and Ag(I) have been employed in de-
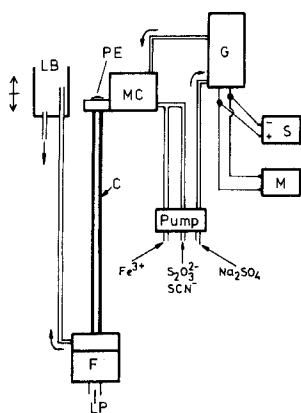


**Fig. 7.10** Experimental set-up for catalytic determinations with electrochemical  generation of the catalyst  as employed for determination of iron  (for further details,  see text). (Reproduced  from [11] with permission of Elsevier).

veloping a large number of determinative methods based on catalytic (determination of $Fe^{3+}$), inhibitory (EDTA), activating ($Zn^{2+}$ and bipyridine) or reactivating ($Cd^{2+}$) effects.

Aoki and Munemori [12] approached the photometric and fluorimetric determination of gases by application of automatic continuous mixing methods with intermittent introduction of the sample and the use of the set-up depicted schematically in Fig. 7.11a, the most important part of which is a dual-tube system (shown in greater detail in Fig. 7.11b) consisting of an inner microporous polytetrafluoroethylene (PTFE) tube wrapped by another tube of the same material. The particular configuration shown is used in the determination of free chlorine in water, which involves pumping the sample and 1 M HCl solution to the merging point (M) and then into the inner tube ($T_1$) of the separation unit. A 0.1 M NaOH solution is pumped through $P_2$ into the outer tube ($T_2$) of the separator. Molecular chlorine generated as a result of the merging between the sample and the HCl solution diffuses through the microporous tube and is converted into $ClO^-$ upon dissolution in the NaOH stream circulating through $T_2$, subsequently reaching the flow-cell of a UV detector, where it is monitored at 290 nm.
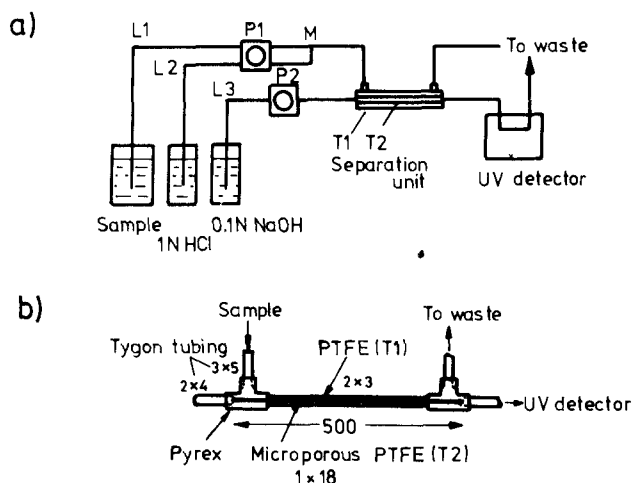


**Fig. 7.11** (a) Instrumental design of a photometric (fluorimetric) gas analyser. (b) Separation unit featuring two concentric PTFE tubes of different porosity. (Reproduced from [12] with permission of the American Chemical Society).

The same type of membrane and a similar configuration are used in the fluorimetric determination of ammonia in water based on the reaction of this base with o-phthalaldehyde (OPA) in the presence of alkaline 2-mercaptoethanol (MCE) to yield a fluorescent adduct [13]. In this case, the merging of the

sample solution  with a  stream of 1 M NaOH gives rise to the formation of
NH₃, which diffuses through T₁ into T₂, where it reacts with the OPA–MCE mix-
ture, the development of this indicator reaction being monitored at $\lambda_{ex}$ = 370
nm and $\lambda_{em}$ = 486 nm. Some distilled water is circulated through T₂ for 5 min
after each measurement and the reading thus obtained is taken as a reference
to be subtracted from that provided by the sample [14].

   Blaedel and Hicks [15] developed several methods for the determination of
various enzymes and substrates by use of a dual-beam spectrophotometer in
which the reference and sample cells are located in series (kinetic methods)
or in parallel (differential methods). They determined glucose by a kinetic
method implemented with the configuration depicted in Fig. 7.12a and the aid
of the enzymes glucose oxidase and peroxidase, the former to promote the aer-
ial oxidation of the substrate to gluconic acid and the latter to facilitate
the oxidation of o-tolidine to benzidine (monitored at 635 nm) by  $H_2O_2$ gener-
ated in the previous reaction. The reagent and the sample stream are set in
motion by gravity flow and with the aid of a peristaltic pump, respectively,
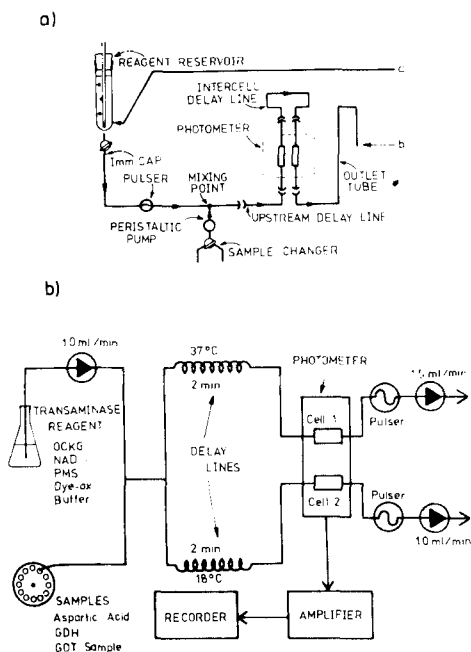


**Fig. 7.12** Analysers  for (a) direct and (b) differential  kinetic de-
terminations.  The determination carried out on (a) (glucose) is based
on  two measurements made at two  different times; that performed with
(b) (transaminases) relies on the simultaneous measurement of a signal
increment  arising from the difference in reaction rate resulting from
a difference in temperature between the two channels. (Reproduced from
[15] with permission of the American Chemical Society).

and meet at a merging point. A three-way stopcock located prior to the pump allows switching from sample to sample with no introduction of air or disturbance to the flow of sample. The pulser situated before the merging point is intended to increase the efficiency of reagent-sample mixing and to facilitate the flushing of the measured sample by a fresh sample. The reacting mixture yields signals of different intensity on passage through each of the cells (different reaction times); this permits kinetic measurements of the form $\Delta S/\Delta t$ to be performed. This method has been applied to the determination of glucose in blood [15].

The instrumental design illustrated in Fig. 7.12b enables the user to carry out differential measurements for the determination of enzymes. The chemistry associated with the determinative procedure is as follows:

$$\text{amino-acid} + \alpha\text{-ketoglutaric acid} \rightleftharpoons \text{keto-acid} + \text{glutamate} \tag{1}$$

$$\text{glutamate} + NAD^+ \rightleftharpoons \alpha\text{-ketoglucarate} + NADH + NH_4^+ \tag{2}$$

$$NADH + \text{oxidized dye} \rightleftharpoons NAD^+ + \text{reduced dye} \tag{3}$$
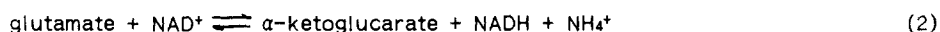
The enzyme sample (serum or tissue homogenate) is diluted in GDH (glutamic dehydrogenase) and buffered amino-acid, and introduced at a constant flow-rate of 1 mL/min until meeting a reagent stream containing the remaining ingredients required for the reaction to develop, namely $\alpha$-ketoglutarate, a transaminase (GXT), nicotinamide adenine dinucleotide (NAD⁺), phenazine methosulphate (PMS) and the dye 2,6-dichlorophenolindophenol. The mixture resulting from the merging is split into two lines thermostated at 37 and 18°C, respectively. After a residence time of 2 min, the content of each line flows through its corresponding cell. The absorbance difference between the two signals obtained is then measured and recorded. The use of calibration standards and a suitable computer program allows readings to be reflected on the recorder chart directly in enzyme units [16].

## 7.3 STOPPED-FLOW CONTINUOUS MIXING METHODS

Under this heading are discussed those methods in which for some reason or another the flowing stream is halted somewhere along the system, either to change the aspiration probe from the sample solution to the reagent or wash solution, to effect sample changeover or to allow sufficient time for the indicator reaction to develop. Stopped-flow continuous mixing methods can be implemented in three different manners depending on the aim of the flow interruption: by inserting the sample without injection, on the basis of fast kinetics and by means of the so-called *stopped-flow/unsegmented storage analyser.*

### 7.3.1 Sample Insertion without Injection

This analytical methodology, described by Riley *et al.* [17], who original-

ly gave it the unfortunate name of 'FIA without injection', is an interesting alternative both to FIA and to automatic segmented methods. The typical insertion or injection of the sample by means of a valve is replaced here by a peristaltic pump stopped and started at strictly controlled intervals. A kinematically controlled probe aspirates a given sample volume through a steel tube dipped into the sample solution, after which it is raised and the pump is stopped. The probe is then immersed in the reagent reservoir and the pump is restarted. The reacting plug is led to the detector via the reaction coil. As no air is aspirated in this operation and the flow is completely bubble-free (unsegmented).
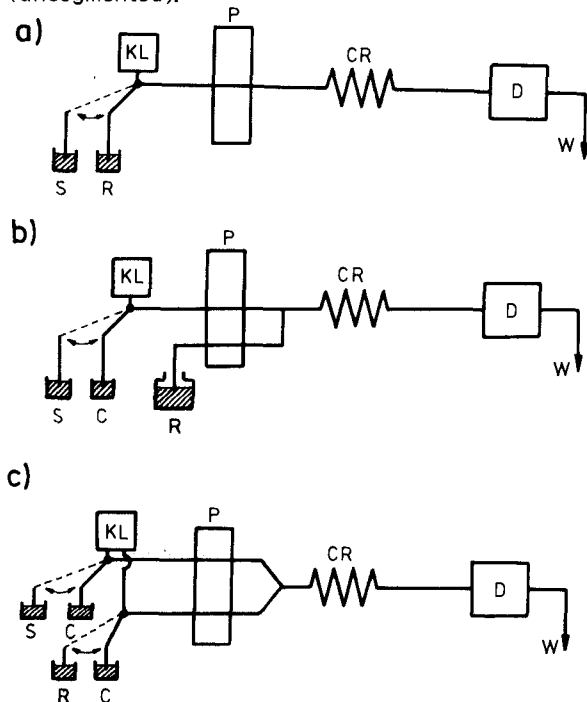


**Fig. 7.13** CDFA systems of different complexity. (a) Straightforward system with sequential aspiration of sample and reagent. (b) The reagent, R, is aspirated in a continuous fashion and mixed with the alternating flow of sample, S, and washing solution, C. (c) Merging zones system with alternate aspiration of sample/reagent and a washing solution. (Reproduced from [17] with permission of the American Chemical Society).

Figure 7.13a shows the general scheme of the set-up used to implement this mode of continuous-flow analysis. The peristaltic pump is connected to a microcomputer-controlled motor. It is interesting that the passage of the reacting plug through the roller-squeezed pump tube results in no increase whatsoever

in dispersion. A dual-channel system within which the reagent is in continuous motion and meets the sample carrier at a merging point prior to the reaction coil (Fig. 7.13b) is a suitable alternative. Figure 7.13c shows a more complicated set-up based on the merging zones principle: two aspiration tubes are immersed simultaneously in the sample and reagent reservoir and then in that of the carrier. The incorporation of a microcomputer permits the system to be automated as regards both its control and data treatment. According to its creators, this analytical mode, known as *controlled dispersion flow analysis* (CDFA), offers a number of major advantages, namely:

(a) Decreased sample consumption as a result of avoiding the use of a triple sample volume to flush the previous sample from the injection valve loop, which is a great asset in clinical chemistry.

(b) Valve leakage due to heavy workloads in hospital laboratories is minimized.

(c) The technique is suitable for joint use with various FIA modes (e.g. merging zones). The microcomputer can be programmed to stop the flow when the reacting plug reaches the detector and hence to apply the stopped-flow mode, particularly suitable for kinetic-enzymatic determinations.

(d) The results obtained are comparable in precision to those provided by an injection valve.

The usefulness and advantages of this technique have been demonstrated by its proponents in the determination of real samples, both clinical and pharmaceutical. Several other configurations similar to those described above have been used for determination of albumin, with a simple set-up similar to that depicted in Fig 7.13a, and of triglycerides and theophylline, by use of a configuration resembling that of Fig. 7.13c and involving merging zones and stopping of the flow at the flow-cell in order to carry out kinetic measurements [18]. Both are discussed in detail in the Chapter 10.
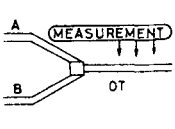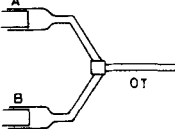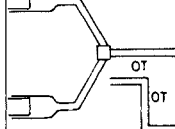
## 7.3.2 Methods based on fast kinetics
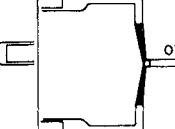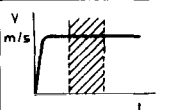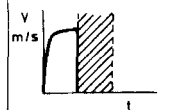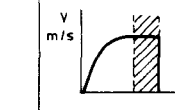
Time is a distinct feature of kinetic methods as compared with equilibrium methods. For a kinetic method to be successfully applied it is in fact essential  to strictly control variables such as the delay time or the measurement time.

The method used to determine the rate of a given reaction obviously depends on its half-life. As a rule, reactions with half-lives shorter than 10 s are classed as fast. Such reactions involve specific instrumental requirements as regards both their study as such (structural elucidation, determination of rate constants) and their use for analytical purposes.

The chief problem with methods based on fast kinetics lies in achieving

instantaneous and reproducible mixing of the reactants. Mixing devices de-
signed so far have been aimed at lowering the level of reaction half-lives to
which this methodology could be appplied, at decreasing the volume of reactant
solution needed and at improving the design of the system controlling the over-
all operation. Figure 7.14 shows representative schemes of the configurations
used to implement the most important mixing methodologies described to date:
continuous-flow, accelerated-flow, stopped-flow and pulsed-flow methods.
Others such as relaxation methods have a narrower scope of application, namely
reversible systems.

| | CONTINUOUS FLOW | ACCELERATED FLOW | STOPPED FLOW | PULSED FLOW |
|---|---|---|---|---|
| SCHEME | | | | |
| MINIMUM VOLUME | $3 \cdot 10^{-3}$ s | $5 \cdot 10^{-3}$ s | $3 \cdot 10^{-3}$ s | $4 \cdot 10^{-5} - 10^{-4}$ |
| REQUIRED | 25 – 250 mL | $\leqq 1$ mL | 0.2 mL | 4 mL |
| MAXIMUM FLOW VELOCITY | 4 m/s | $\geqq 10$ m/s | 3 m/s | 2–9 m/s |
| FLOW VELOCITY AS A FUNCTION OF TIME | | | | |

**Fig. 7.14** Mixing methods for fast reactions. The figure shows the
half-lives to which they can be applied, the reagent volume used, the
maximum velocity attained by the flow and the measurement zone used in
terms of such a velocity.

### 7.3.2.1 Continuous-flow methods

These were the earliest to be developed (Hartridge and Roughton, 1923). By
means of gas or hydrostatic pressure or with the aid of a peristaltic pump,
the solutions containing the reactants are driven to a merging point or mini-
zone from which they are led to an observation tube (OT) along which is moni-
tored the signal yielded (absorbance) at different reaction times. As the re-
action progresses simultaneously to the same extent at every point along the
OT, measurements need not be made so quickly as in other cases. Reactions with
half-lives as short as $3 \times 10^{-3}$ s can be dealt with by this type of method, the
greatest shortcoming of which is the increased consumption of reactant solu-

tion (circulated at a speed of 4 m/s) in each determination. The distance between the merging point and the first observation point, I, is dictated both by the reaction half-life, which includes the 'dead time' elapsed between passage of a given element of fluid through the mixer and the first observation point, and by the efficiency of the mixing system —ideally, this should be no less than 95%. The latter factor is linked to the reactor geometry, the flow-rate and the viscosity of the flowing solution, which should circulate under a turbulent regime (the Reynolds number should be greater than 5000.)

### 7.3.2.2 Accelerated-flow methods

In this type of method the reactants are held in two hypodermic syringes which are unloaded quickly and simultaneously. The solutions are accelerated from zero velocity to a given value prior to evacuation. The system features a single observation point located at a distance of 7 mm from the merging point. The maximum velocity attained is 10 m/s. As the dead time involved is $7 \times 10^{-4}$ s, the method can be applied to reactions with half-lives equal to or greater than 0.5 ms.
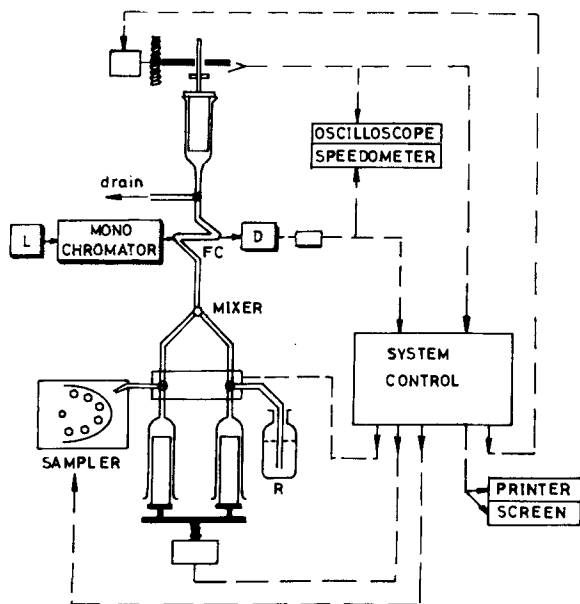
### 7.3.2.3 Stopped-flow methods

The typical system whereby stopped-flow methods are implemented consists basically of the same elements used in the methods described above plus a stop syringe located after the observation tube which halts the flow once this has been accelerated. The above-defined dead time includes a new component here: the so-called 'stop time', i.e. the interval elapsed from the moment that the syringe plunger reaches its retainer to that in which the enclosed fluid is effectively brought to a standstill —absence of shock waves. This time should be much shorter than those of mixing and transport in order that the overall mixing time may be reasonably short. During this period, the flow velocity varies from zero when the system is started to a maximum, $v_{max}$, as in the previous case. The monitoring of the signal as a function of time is started immediately after the stop. The light trajectory can either be perpendicular to the OT, as above, or run parallel to its axis if it has a different configuration. The reactant volumes used are small (0.2 mL) and the maximum velocity attained is 3 m/s. The scope of application extends to reactions with half-lives as short as $3 \times 10^{-3}$ s.

Stopped-flow methods are by far the most popular and frequently used in studies involving fast reactions [19-29]. In fact, several European and American firms supply dedicated instruments or accessories to be fitted to commercially available spectrophotometers.

In Fig. 7.15 is shown the scheme of an automatic analyser involving mix-

ing, transport and measurement by the stopped-flow technique and spectro-
photometric monitoring of the signal. The microcomputer used allows control of
the instrument operation as well as data acquisition and treatment. The mechan-
ical system loads the two liquids and unloads them into the mixing minichamber
in conjunction with the three-way valves, which are duly switched for each
operation. A sampler permits automatic sample changeover between injections.
The flow-cell, T-shaped, lies in the light path of a spectrophotometer. The
stop syringe takes in the mixed solution. The dead time elapsed between thrust
and stop of the plunger is controlled by means of a retainer, the height of
which is regulated with a micrometer screw. The impact of the plunger on the
retainer gives rise to an electric signal that is received by the computer,
which in turn starts collecting the signal from the detector as a drain valve
sets the instrument ready for a new injection. Alternatively, both signals can
be received by an oscilloscope or a speedometer. In non-automated models, the
dual injection is carried out pneumatically or hydraulically and the position
of the retainer is fixed by the turn of the micrometer screw. An electric
switch system fitted to the retainer must always be used to ensure that signal
collection is started accurately as ordinary recorders do not offer suffi-
ciently fast responses.



**Fig. 7.15** Automatic stopped-flow analyser with photometric detector.

### 7.3.2.4 Pulsed-flow methods

This type of method was originally developed by Gerisher (1965 and 1971), who called it 'continuous-flow method with integrating observation' (CFMIO), and later improved by Margerum and co-workers in 1980 [25,26]. It is implemented with the aid of syringe system similar to that used in stopped-flow methods, but with a very short pulse duration. Reagent consumption is only 4 mL and the maximum flow velocity attainable ranges between 2 and 9 m/s. The most outstanding feature of the set-up utilized is 'integrating observation', which involves placing both the mixing zone (front part of the OT) and the remainder of the observation tube in the light path of the sensing system. This mode calls for very efficient mixing over a relatively small area of the OT compared with its length, which is not feasible by merely using two separate conduits leading the reactants to the entrance of the OT, where they merge. The solution to this problem lies in employing a radial mixer/observation cell. Seven channels per reactant unload their contents alternately at the front of the observation tube. All seven radial channels are connected to two circular conduits filled with the reactant solutions, which results in highly efficient, turbulent mixing. As the two syringes are unloaded, the reactants flow through the channels and meet at the OT.

The detection technique most commonly used with methods based on fast kinetics is photometry (either in its conventional form or with multi-detection systems such as image detectors) and, to a much lesser extent, fluorimetric and electroanalytical methods.

The data collection system used is of paramount importance in these methods as the detector provides a large number of data in an extremely short time; thus, the collector, usually a micro- or minicomputer, must be highly responsive to time. The subsequent treatment of the collected data can be very different in nature depending on the particular aim pursued.

### 7.3.3 Stopped-flow/unsegmented storage analysis

This is a continuous flow mode [27] based on an adaptation of the stopped-flow assemblies originally designed to study and use reactions with fast kinetics. The essential modification with respect to the methods described above is the incorporation of a storage coil between the mixing unit and the flow-cell to prevent the reacting mixture from reaching the detector in too short a time, which allows this mode to be applied to reactions attaining equilibrium in times of the order of a few seconds (or minutes).

Figure 7.16 shows the scheme of a prototype for automatic stopped-flow/unsegmented storage analysis (SF/USA). The microcomputer of the system controls the simultaneous injection of sample and reagent with two syringes via a dual

three-way valve. A suitable mixer brings both solutions into contact. The re-
acting mixture passes through the coil (store) and reaches the detector flow-
cell, which is controlled by the microcomputer, which in turn captures and
processes the absorbance signals generated and transduces the results to a
printer, provided that the instrument is previously calibrated with standard
samples.

Normally, each turn of the coil stores the contents for one injection. The
system depicted in Fig. 7.16 requires six aliquots for the coil to be flushed
from the previous sample, and a seventh to be used for measurement and which is
followed by another aliquot intended to isolate it from the effects of the next
sample to be injected. The scheme shown in Fig. 7.16 (bottom) represents two
sequential situations: (1) after injection of the six flushing aliquots, three
of which have by that time passed the detector unmeasured, plus the measurement
aliquot (sample A) and the isolating aliquot; and (2) upon injection of the
next sample (B), when the previous sample has not yet been measured. The time
elapsed in aliquoting, mixing and injecting the samples into the storage coil
is 1.6 s. Hence, the measurement aliquot takes 9.6 s (6x1.6) to traverse the
length of the coil and reach the detector.
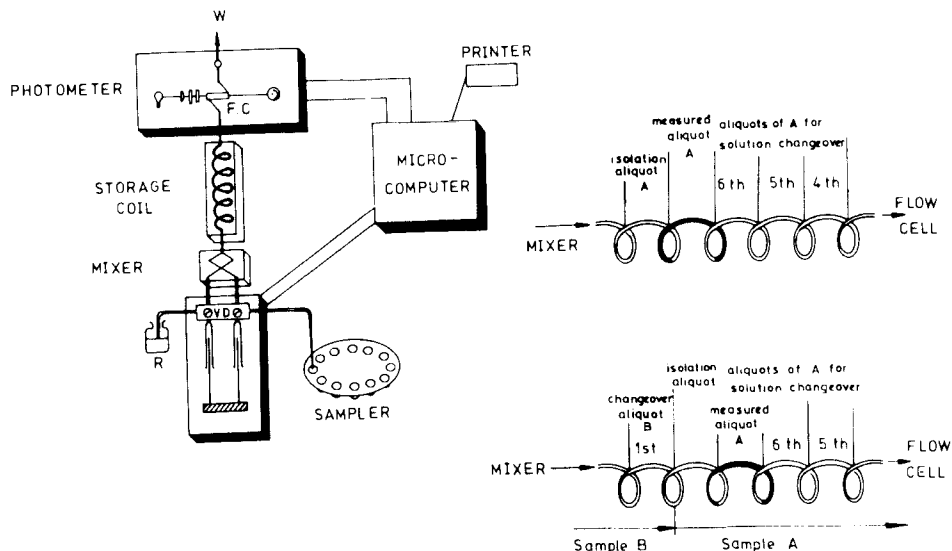


**Fig. 7.16** Stopped-flow/unsegmented storage analyser (SF/USA) and en-
larged sections of the storage coil.

In this manner, Malmstadt *et al.* [27] applied typical stopped-flow tech-
nology to the analysis of a series of samples on the basis of reactions with
normal kinetics. According to its proponents, SF/USA offers significant advan-
tages over SFA and FIA:

(a) The precision is of the order of 0.1%, i.e. ten times higher than that
achieved by other automatic continuous methodologies.

(b) It is compatible with reaction-rate methods, even with those involv-
ing measurements in the fraction-of-a-second range.

To demonstrate the latter feature, they have applied their method to a
set of systems with very different reaction rates. They developed a method for
the determination for Fe(III) based on the reaction of this ion with ammonium
thiocyanate, which is monitored over 1 s. As the reaction is completed in 10
s, the storage coil can hold six sample aliquots.

The determination of protein nitrogen by Berthelot's reaction, which is
slow, has been speeded up by application of SF/USA with a 12-turn storage coil
and measurement of the absorbance at 620 nm after reaction for 20.8 s (i.e.
before completion). In a similar way have been developed a method for determi-
nation of phosphorous by formation of the well-known heteropolyacid —subse-
quently reduced with ascorbic acid—, using a 12-turn coil (travel time 21.2
s), and a method for ascorbic acid based on its oxidation with 2,6-dichloro-
phenolindophenol, a reaction which takes 30 s to complete, so that the dalay
time resulting from the 18-turn coil used is 18 s.

The SF/USA methodology is therefore applicable over a wide range of re-
actions rates. Moreover, it affords sampling rates above 200 samples/h in
every instance and features low sample consumption and outstanding reproduc-
ibility.

## 7.4 CONTINUOUS-FLOW TITRATIONS

This type of titration overcomes one of the most serious drawbacks of ti-
trimetric analysis, namely the long operational time involved. As the results
of all the continuous-flow titrimetric determinations developed to date are
obtained by monitoring of the analytical signal as a function of time, this is
a variable of paramount importance and therefore calls for strict control. The
flowing stream into which the sample is inserted or with which it is merged
can be either stopped after each titration or kept circulating between consecu-
tive samples, thus giving rise to different modes, all of which result in a
considerable decrease in the time usually needed for conventional titrations.

### 7.4.1 Without halting of the flow between samples

Under this heading are included the so-called 'FIA titrations' or 'scale

expansion techniques', proposed by Ruzicka *et al.* [28]. Such techniques, exemplified by an acid-base (NaOH-HCl) photometric titration, involve the injection of a large analyte volume (200-500 µL) into a reagent stream containing an indicator system. The sample plug gives rise to a broad peak upon arrival at the detector. There is a given absorbance value represented by one point in the rising portion and another in the tail of the peak which correspond to complete neutralization between the base and the acid, i.e. at which the titrant and titrand are in a stoichiometric ratio, as in conventional titrations —hence the name. It has been demonstrated that there is a linear relationship between the width of the peak obtained and the logarithm of the analyte concentration.

This type of titration can be implemented (Fig. 7.17) by intercalating into a single-channel FIA system a gradient chamber (partial mixing chamber) or simply a reaction tube (high-speed FIA titrations) [29].
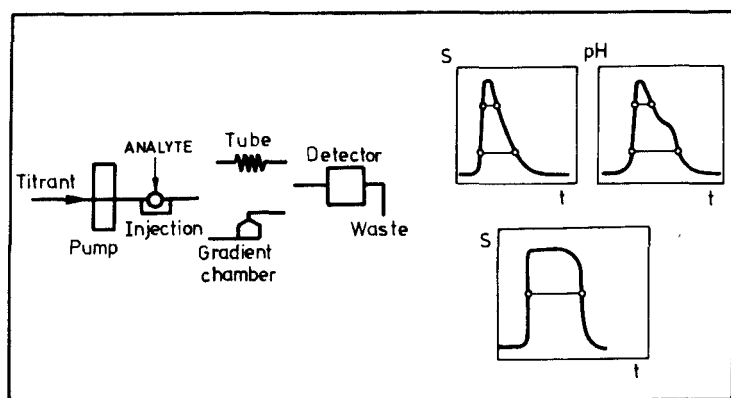


**Fig. 7.17** Manifolds used in the development of normal (gradient chamber) and high-speed (tube) FIA titrations, and their corresponding measurements and recordings.

Pardue and Fields have made a deep theoretical study of these systems [30, 31], questioning the suitability of giving this FIA mode the name "titration", as it does not conform to the typical features of conventional titrations. In fact, (a) the sample and reagent react in non-stoichiometric amounts, (b) the dynamic continuous flow system involves sample or reagent losses by mass flow rather than through reaction, and (c) the resuls obtained with and without a reagent in the flowing stream are similar, so that no chemical reaction is required for a calibration graph to be run.

These reasons led Pardue and Fields to consider that this type of titration is actually another variable-time kinetic method insofar as it is based on the measurement of a time increment, $\Delta t$, between two preselected reference points located at the same height from the baseline in the rising and falling portions of an FIA peak yielded by a physico-chemical process which has attained neither physical equilibrium —there is some mass flow between the sample plug and the regent solution— nor chemical equilibrium —the reaction is still incomplete.

Although the argument of Pardue and Fields is quite valid if one considers that no equivalence point or physico-chemical equilibrium is attained in FIA titrations, the proposal of Ruzicka *et al.* is supported by a number of facts:

(a) The argument of Pardue and Fields that similar results are obtained in the presence and absence of a chemical reaction is not strictly valid as this statement was inferred from the use of an acid-base indicator, which undoubtedly reacts (changes in colour) with the basic medium in which it is dissolved. Strictly, there would be no chemical reaction as such if the detector used (potentiometer, atomic absorption spectrometer, etc.) directly measured the analyte concentration.

(b) It should be emphasized that a reagent stream is chemically much more versatile than a water stream, whose only appreciable effect is sample dissolution.

(c) As the reagent is circulated at a constant flow-rate, the time increment used as an analytical signal can be related to a reagent volume in much the same way as in classical titrations, although such a volume also varies with the preselected level at which the signal is measured.

(d) The resemblance of the methodology of Ruzicka *et al.* to ordinary titrations becomes more apparent if one considers that the tailing portion of the FIA peak is very similar to a titration curve (plot of pH, mV, etc., against the volume of titrant added).

(e) Finally, there are two points, one in the rising and another in the falling portion of the peak, at which the analyte and reagent coexist in a stoichiometric ratio. The time increment between these two points reinforces the similarity between this methology and conventional titrations.

Stewart and Rosenfeld [32] looked at the subject from a different angle. They concluded that the use of volumes as large as those employed in this FIA mode permit the linear ranges of the calibration graphs to be significantly widened. As a result, they proposed the denomination 'scale-expansion techniques' as an alternative to 'FIA titrations'.

### 7.4.2 With halting of the flow between samples: kinetic titrations

Pardue and Fields [30,31], who established differences between  various

types of titration, took the automatic potentiometric titration system pro-
posed by Blaedel and Laessing [33] as a representative example of kinetic ti-
trations. The system in question included a tubular platinum electrode (TPE)
as indicator which, in conjuction with an appropriate reference electrode,
allows a potential that is a function of the concentration of electroactive
species at the electrode to be obtained. Such a potential is taken as a refer-
ence to control the pumping rate of the titrant, whereas the sample flow-rate
is kept constant, so that its concentration is a function of the titrant
flow-rate at the equivalence point. The potentiometer, connected to the cen-
tral unit, continuously compares the cell potential with that corresponding to
the end-point. The assembly used (Fig. 7.18) is very simple and requires no
burette or standardized titrant, a standard calibration sample being used in
its place. The results are usually delivered in digital form.
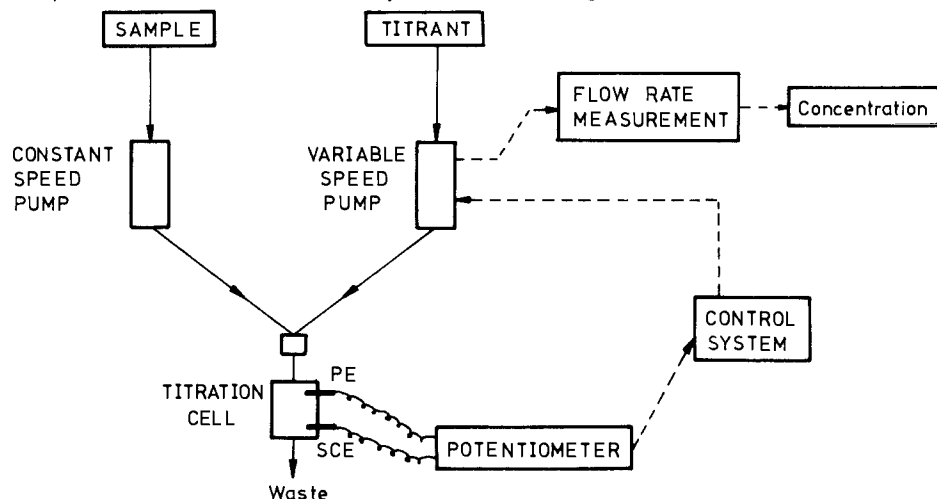


**Fig. 7.18**  Scheme  of a typical instrument for kinetic titrations fea-
turing constant flow-rate of sample and variable of reagent.

More recently, Abicht [34] proposed a 'controlled dynamic titrator' which
operates inversely to the above-described system, so that the titrant  flow-
rate is maintained constant while that of sample changes with time. Figure
7.19a shows the titrator. The liquid samples to be measured are placed on the
sampler. Every 2 min the sampling burette takes a new sample and unloads it
into the mixing cell as it sends a suitable 'order' for the titration to be
started. The flow of titrant is activated simultaneously with that of the sam-
pling burette. A second pump supplies the cell with a constant flow of sol-
vent, which mixes homogeneously with the sample and titrant. After a short
reaction tube, the mixture enters the detector flow-cell, from which it is

subsequently driven to waste. Titration times are printed alongside with their corresponding number of the sample. Figure 7.19b shows a typical plot in which the shortest titration time corresponds to the most concentrated sample, because as $v_m = kt$, then $N_m = N_v v_v/kt$, i.e. the sample concentration is inversely proportional to the duration of the titration; all other parameters are kept constant. Photometric, potentiometric and voltammetric detectors used with this titrator have yielded good results.

a)
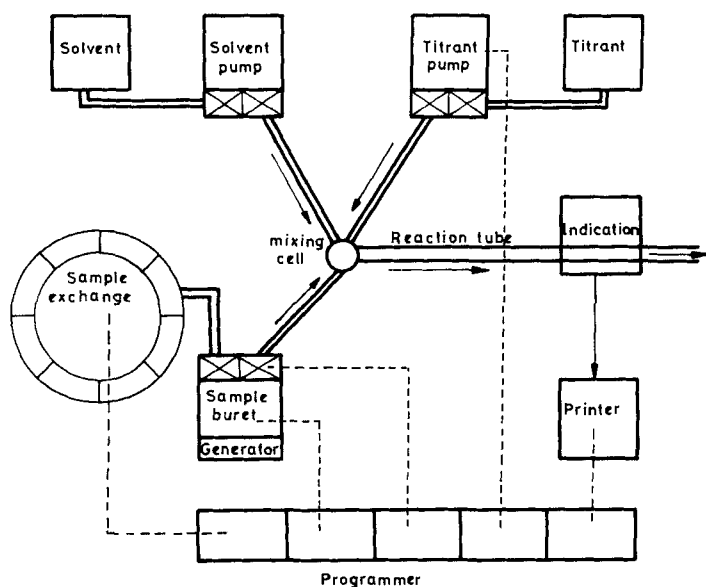


b)
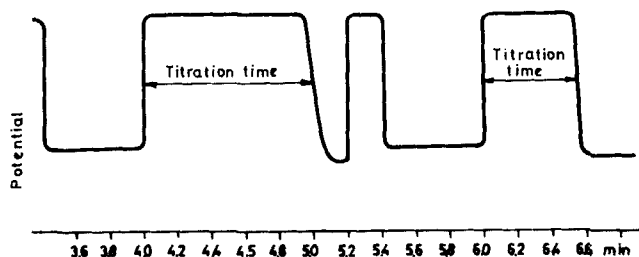


**Fig. 7.19** (a) Dynamic titrator featuring a constant flow-rate of the titrant and a variable flow-rate of the of sample. (b) Typical forms of measurement and recordings. (Reproduced from [34] with permission of Elsevier).

Nicholson proposed a differential potentiometric titrator involving two indicator electrodes for the automatic control of processes in industrial plants [35]. As can be seen from Fig. 7.20, the sample and reagent streams are split and led to two half-cells via capillary tubes adjusted to provide slightly different titrated fractions. The potential difference ($\Delta E$) between the two indicator electrodes is transmitted to a control and detection system (D) which regulates the flow of titrant in an automatic fashion by means of valve V, thereby maintaining the preselected $\Delta E$ between the two ends of the cell. The speed of titrant addition, reflected by the flow meter (M), is a measure of the sample composition. An evaluation of the instrument carried out by the titration of dichromate with iron(II) revealed that the conditions to be used must be carefully selected. Thus,  stable electrode responses are only obtained in the zone where Fe(II) prevails, and not in that where dichromate prevails over the former as the process determining the potential obtained in such a zone is irreversible. This method therefore has limited application in the control of slow reactions.



D   Detector controller system
M   Flowmeter
V   Valve
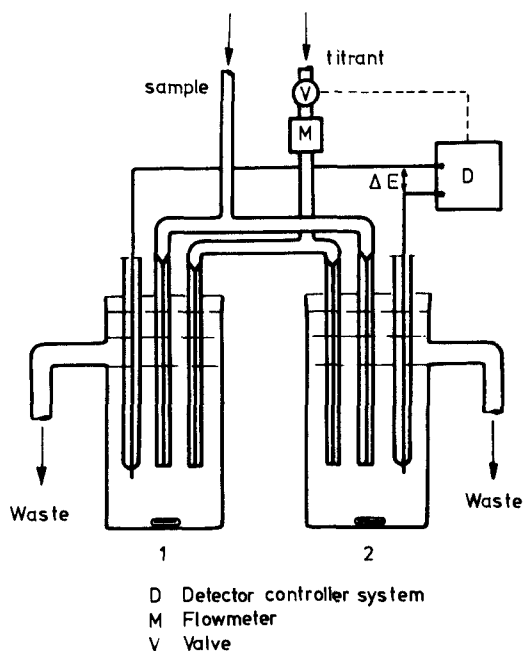
**Fig. 7.20** Differential potentiometric titrator. (Reproduced from [35] withe permission of the American Chemical Society).

A completely different conception of flow titrations, although suitable

for inclusion in this section, was introduced by Griepink *et al.*, who developed a flow titrator involving optical detection. They also reported a theoretical model describing both the instrument and the flow that circulates through it from the results obtained in the titration of sulphate with barium. The schemes in Fig. 7.21 illustrate the basis of this titration. Samples from a turntable are mixed with a set-point solution consisting of barium sulphate solution, barium ions and dimethyl sulphonazo III (DMSA), and fed to an 8-mL cell to which the titrant is dispensed. The excess solution in the cell is continuously pumped to waste. The reaction involved in the determination,

$$Ba(DMSA) + SO_4^{2-} \rightleftharpoons BaSO_4 + DMSA^{2-}$$
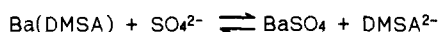
is monitored by the sensing system depicted in Fig. 7.21a. This includes a bulb emitting white light (I) which passes through two filters and is delivered at two wavelengths: $\lambda_1$ [isobestic point for Ba(DMSA) and DMSA$^{2-}$] and $\lambda_2$, at which both substances have different molar absorptivities. The consistency between simulated and experimental responses confirms the suitability

a)

b)
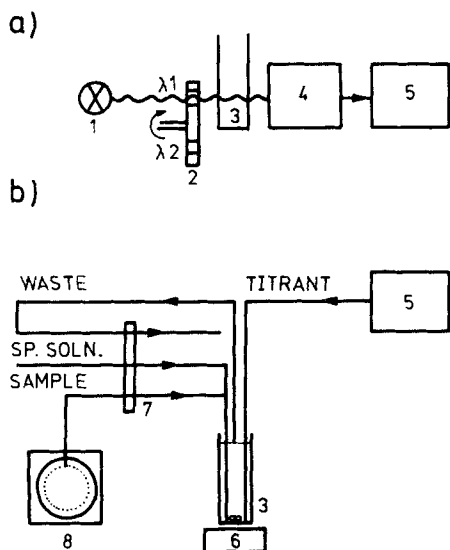


**Fig. 7.21** (a) Detail of automatic titrator with photometric detection system; (b) general configuration of the titrator. (Reproduced from [36] wih permission of Elsevier).

of the proposed models [36,37]. Determinations are carried out in a maximum

time of 2 min, with wash periods of the same length in between [38]. The results obtained in the application of this method to real samples (various types of water) indicate its suitability [39].

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# 8
## Automatic batch analysers

## 8.1 INTRODUCTION

Discrete or batch automatic analysers (DAA) are characterized by discontinuity: each of the samples assayed preserves its integrity in the cuvette or cup where it is held and mechanically transported to the detection zone. Unlike in continuous automatic analysers (CFA), the sample is transported by means of a flowing fluid continuously circulating along the system. However, they do not differ so markedly from robotic configurations: both feature mechanical transport, although in DAAs this is carried out by a conveyor belt or a turntable, whereas in robot stations it is performed by the minirobot mimicking the actions of a human operator.

Despite the frequent use for a long time now of sample trains to reduce human intervention, the earliest DAA models were introduced after Technicon Autoanalyzers (SFA) in response to the need for enhanced performance in some fields —particularly in clinical chemistry.

In fact, DAAs feature a number of advantages over continuous analysers:

(a) They allow the rapid processing of a large number of samples and the convenient determination of several analytes in the same sample.

(b) They are more versatile, i.e. more readily adapted to different needs without much alteration.

(c) Because of their inherent characteristics —samples are physically isolated from one another— the risk of sample carryover is much lesser and arises from the sampling system and the use of paddle agitation; a common element is brought into sequential contact with the samples and reaction mixtures, which can give rise to cross-contamination unless an efficient clean-up system is employed.

(d) They allow the use of corrosive fluids (e.g. organic solvents, concentrated acids) impossible with continuous analysers on account of the risk of the system connections and walls being attacked.

However, DAAs have some disadvantages or shortcomings that should not be underestimated:

(a) They are generally complex mechanically. In fact, they are cumbersome

to maintain as they require skilled workers even for minor repairs. Moreover, current commercially available DAAs are highly computerized —built-in microprocessors play a major role in their design and functioning, which adds to the aforesaid complexity.

(b) Despite their versatility, their basic configurations are difficult to change. Thus, in FIA systems, the replacement of some component or the incorporation of an another module —from a straightforward heater to a sophisticated continuous separation unit— is a relatively easy task. Hence, research on and the development of automatic methodologies is easier by continuous methodologies for laboratories without the infrastructure required to incorporate the latest advances in micromechanics and microelectronics.

(c) They are generally much more expensive than continuous analysers.
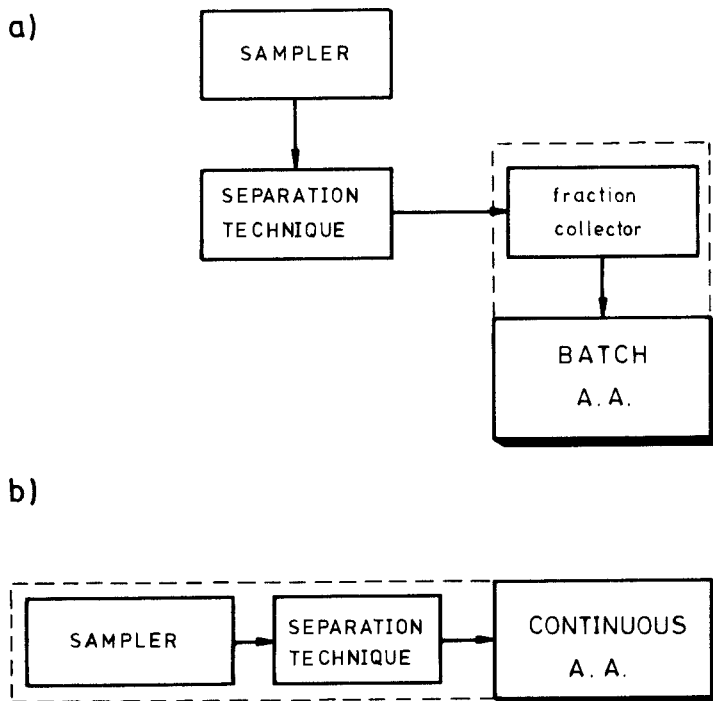


**Fig. 8.1** Ways of incorporating separation techniques into (a) batch and (b) continuous automatic analysers.

(d) Because of their configuration, they do not allow the on-line incorporation of analytical separation techniques, unless these are implemented on self-contained automated units independent of the analyser. Figure 8.1 illustrates the different manners in which a separation process (sample treatment) can be incorporated into the two above-mentioned types of analysers.

Analytical processes performed by DAAs are similar to those carried out manually. Hence, they allow readier adaptation of manual methods than do continuous analysers insofar as the latter require stricter optimization of the different chemical and physico-chemical variables involved. As can be seen from Fig 8.2, of the three types of automatic analysers, robotic types bear the strongest resemblance to manual configurations.
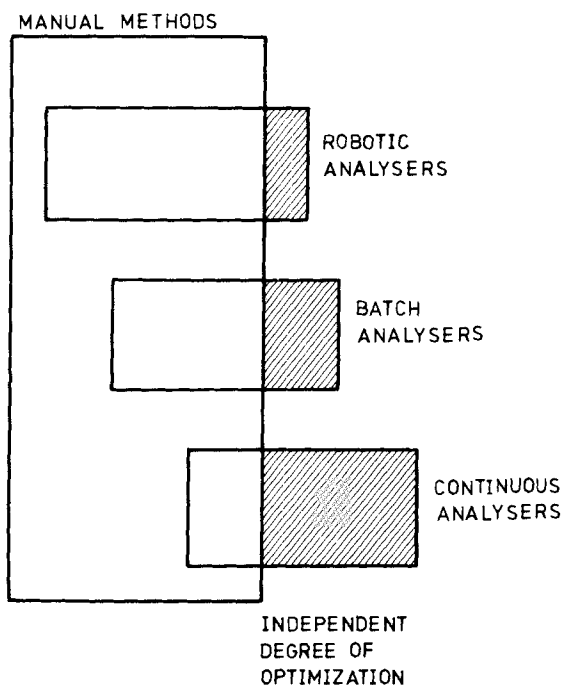


**Fig. 8.2** Adaptation of manual analytical methods to the three basic types of automatic analysers.

## 8.2 CLASSIFICATION OF BATCH ANALYSERS

As can be seen from Table 8.1, the large variety of DAAs currently available can be classified according to a number of criteria:

(a) According to the *manner in which the analytical signal is measured*, one may distinguish between *sequential measurement*, characterized by a fixed time between measurement of samples —this is the commoner case— and *simultaneous measurement*, where data are obtained from all samples at roughly the same time and with the same detector —this is typical of the so-called 'centrifugal analysers', where detection takes place in a parallel configuration: signals are generated in a virtually simultaneous way on passage of the cuvettes at a high speed through the light path of a single photometric detection point, the flow of data being controlled by a microprocessor.

**TABLE 8.1**

Classification of batch analysers

| According to measurement | Serial | |
|---|---|---|
| | Parallel | |
| According to sampling | Manual | |
| | Automatic | In the analyser |
| | | On-line module |
| According to number of analytes | Single-parameter | |
| | Multi-parameter | |
| According to final transfer | With | |
| | Without | |
| According to measuring cell | Number | One |
| | | Several |
| | Material | Disposable |
| | | Non-disposable |
| According to motion | Circular | |
| | Non-circular | |

(b) According to whether *sampling* is automated or not, a distinction can be made between DAAs *with* and *without* *automatic sampling*. The former, usually highly automated, are conceived for the analysis of a large number of samples. They are represented by the batch analysers for clinical determinations such as the Technicon RA-1000 or Union Carbide Centrifichems, and by instruments (gas or liquid chromatographs and electrothermal-vaporization atomic absorption spectrometers) with a sampling module coupled on-line. These configurations, usually featuring automated data acquisition and treatment, can be classed as analysers, although they are more commonly referred to as instruments to emphasize their determinative rather than their automatic aspects.

(c) According to their *capability*, DAAs can be classified into *single-parameter* and *multi-parameter*. The former are designed for the determination of a single analyte per sample and perform a single, pre-programmed operational sequence (reagent delivery, stirring, heating, measurement). The latter can be programmed to determine several analytes in the same sample. Figure 8.3 shows the basic differences between multi-parameter configurations in batch and continuous analysers. In continuous-flow analysis, multi-parameter designs —here equivalent to multi-channel designs— require a precise sample splitting system and a series of reaction and detection modules or systems, one per analyte

to be determined. On the other hand, multi-parameter designs in DAAs, improperly called multi-channel designs, split the samples into aliquots by means of a sampling system; each sample is held in an independent cup according to the methodology implemented for each parameter —a different reagent for each analyte, heating or not, etc. A major difference between the two types of configurations is the occurrence of a single detection point in DAAs.
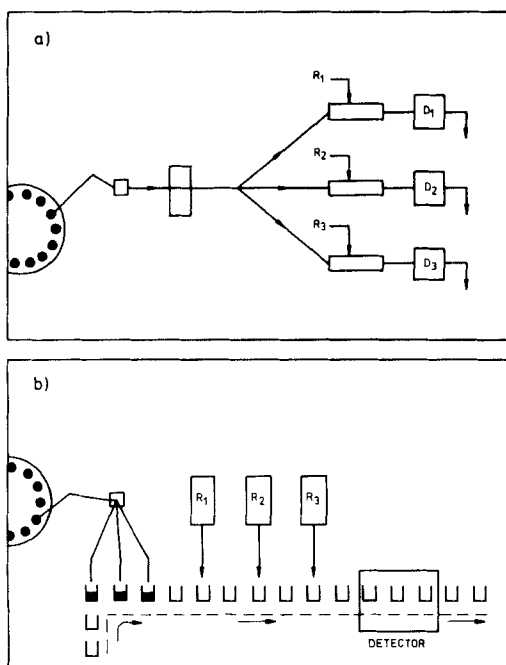


**Fig. 8.3** Scheme of the commonest configurations of (a) continuous and (b) batch analysers for multi-parameter determinations.

(d) According to the *manner in which the sample is transferred* in the final operation, DAAs can be said to function *with* or *without final transfer* (Fig. 8.4). In analysers with final transfer, the reaction mixture is transported to the detection system, where the analytical measurement is carried out in a fixed cuvette. Samples do not preserve their integrity in the analyser and are therefore liable to carry-over. In analysers without final transfer of the reaction mixture, all the stages of the process (sample reception, reagent dispensing, heating, stirring and measurement) take place in the same vessel. This criterion is the basis for the description of the DAAs with automatic sampling described below.
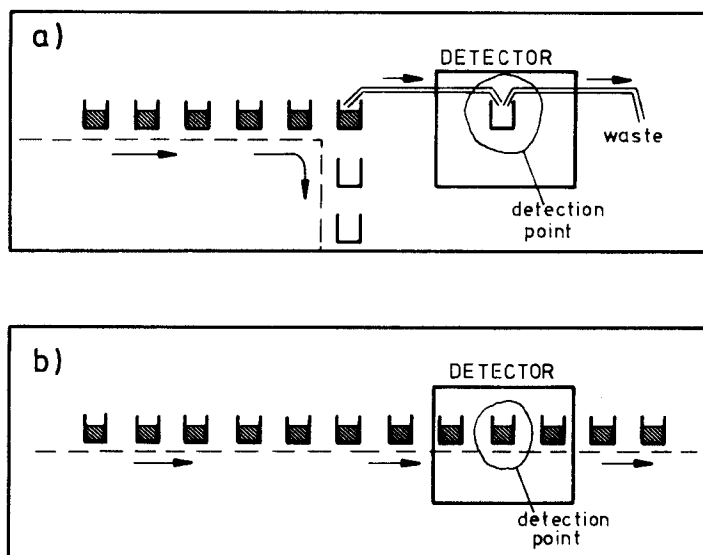
**Fig. 8.4** Batch analysers (a) with and (b) without transfer of the re-
action mixture prior to detection.


(e) Batch analysers can also be classified according to the *measuring cell*
used. Thus, DAAs use a single cell or a number of cells —one per sample or
analyte— depending on whether they involve final transfer of sample or not.
Some analysers with potentiometric detection use selective electrodes for
multi-determinations. A distinction can also be made according to whether the
cuvettes used are disposable or not.

(f) The typical cups used by batch analysers can be moved *circularly* and
*linearly*. In DAAs with circular motion, cuvettes are placed around the peri-
phery of a disc which is rotated like a conventional sampler. This is the way
in which the Technicon RA-1000 and Hitachi 705 operate. Centrifugal analysers
feature a dual circular motion: that of the dosing and that of the analyser
module. In DAAs with linear motion, the cups are displaced along a not com-
pletely linear trajectory including some turns at different angles (90°, 180°
or intermediate values). This configuration affords a large number of cups to
be handled and hence increases the analyser capacity. However, these systems
are much more complex from a mechanical point of view.

In organizing this chapter, DAAs have been classified according to
whether they feature built-in automatic sampling or not. A further distinction

is made in the latter instance depending on whether they include a module coupled on-line for that purpose.

## 8.3 BATCH ANALYSERS WITH AUTOMATIC SAMPLING

This section deals with commercially available analytical processors designed for the analysis of a large number of samples containing one or several analytes in which the sampling system is a non-differentiated part of the system. They are characterized by their flexibility for adaptation to different needs in the determination of a large variety of analytes. Their sole limitations in this respect are the state of aggregation of the sample and the detection system to be used —photometric or spectrophotometric.

In dealing with these analysers, a distinction will also be made according to whether the sample is transferred or not from its original position to the detection system.

### 8.3.1 Batch analysers with final transfer

Vitatron manufacture an analyser called AKES for the automatic determination of enzymes and substrates based on kinetic photometric measurements (see Fig. 8.5). The sample train used is moved linearly. The sample is aspirated in a fixed position from a vial to a cuvette (no. 14) placed on a turntable as a preset volume of diluent is added. There is a single measurement point (cuvette no. 1), where the absorbance is monitored as a function of time. In the immediately prior position, the reagent is added and stirred with a rod, whereas in the immediately following position, the cuvette contents are emptied with the aid of a suction system that sends the solution to a waste bottle. In the next position, the cuvette is washed and again drained by suction. The end of the sample train features a position where the sample being measured at the detection unit can be identified by means of an optical sensor. Thus, the printer receives two signals; one from the microprocessor (the analytical result obtained after processing of the absorbance-data times and correlation with the analyte concentration via the calibration graph) and another from the sample-result matching system.

*Automatic centrifugal analysers* are also batch analysers with final transfer of the reaction mixture prior to measurement. They have become very popular in clinical laboratories in the last few years. The different manufacturers offer a wide a range of models; the Centrifichem Models 400 and 600, manufactured by Union Carbide, are probably the best known and most widely used, so they will be described here as the most typical representatives. It should be noted, though, that there are no significant differences between

different prototypes such as the GeMASAEC (General Medical Sciences Atomic
Energy Commission) and those developed by the Oak Ridge National Laboratory.
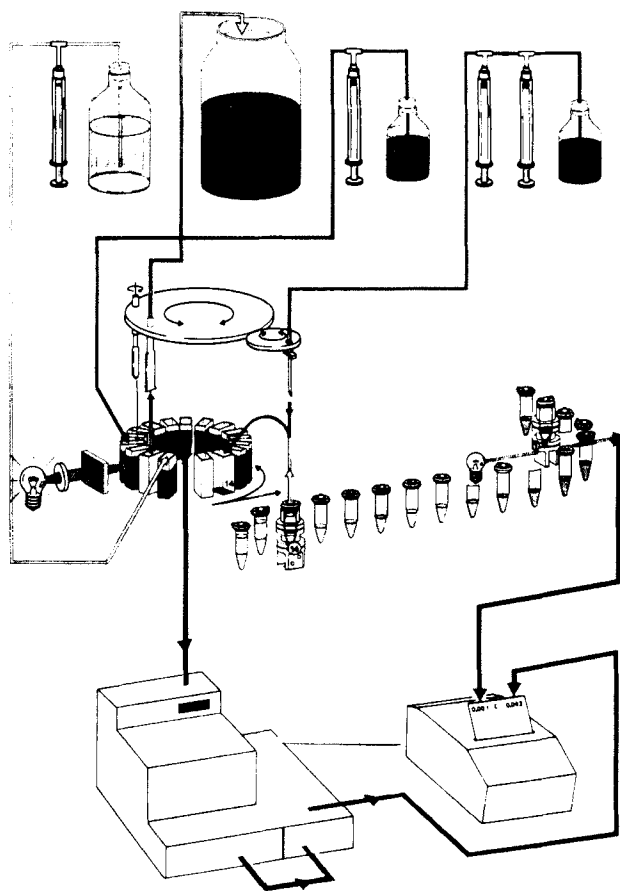


**Fig. 8.5** Scheme of the Vitatron AKES analyser, designed for kinetic
photometric determinations. (Courtesy of Vitatron).

    Centrifugal analysers consist of two separate instrumental units: the
*dosing module*, which dispenses samples and reagents, and the *analyser module*,
where samples and reagents are mixed and the reaction mixture is measured,
generally spectrophotometrically. The most advanced models feature a computer
that controls the operation of the two units and allows the user to program
the system for the determination of a given number of parameters and delivery
of printed results.
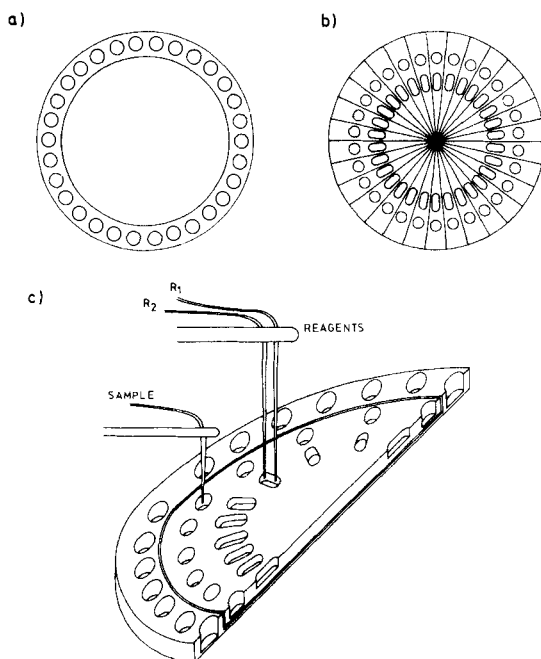
**Fig. 8.6** Scheme of automatic centrifugal analysers. Dosing module:
(a) sample turntable; (b) radial transfer unit; (c) location of the
previous units on sample and reagent dosing.

Figure 8.6 shows the main elements of the dosing module. A sample turn-
table (a) holds samples (serum, urine) on its outermost disc. The transfer
disc (b) is the most important part of the system. It is made of Teflon and
contains 30 radially arranged units (Figs 8.6b and 8.7). Each of these units
has a reagent cavity (a), sample cavity (b) and transfer port (c).

The sample and transfer discs can be coupled. The module rotates by 12°
(360:30) to place the different units sequentially in the pipetting zone. Two
moving pick-up arms connected to high-precision dosing pipettes effect the
transfer. The sample (standard) pipette transfers a preset volume from the
disc microvial to the sample cavity with an intermediate washing operation.
The pipette(s) transfer(s) the reagents from cuvettes not depicted in the
figures to their corresponding cavities. Once the transfer is finished, the
transfer disc is taken manually from the dosing module to the analyser module,
where the assembly is fitted to the rotor and covered with a plastic lid (Fig.
8.7a) to obtain a temperature-controlled chamber. A hydropneumatic system
evacuates the chamber, both for mixing of reactants and for the washing and
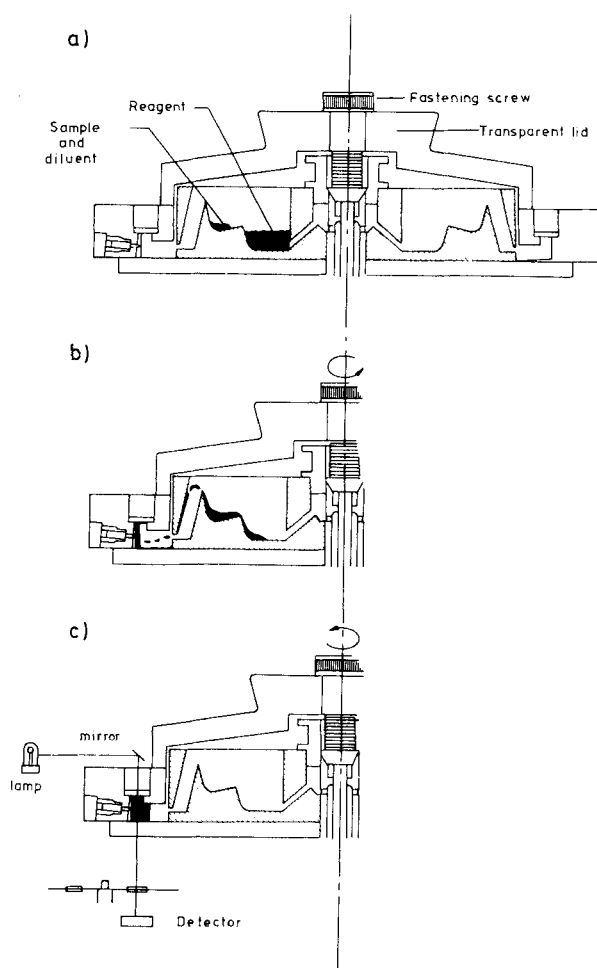
**Fig. 8.7** Scheme of automatic centrifugal analyser. Analyser module: (a) location of the transfer disc; (b) the transfer of the reaction mixture takes place during centrifugation; (c) optical detection system on a single, fixed point.

drying operations following the determination. This disc is spun at 960 rpm and the resultant centrifugal force is sufficient to sweep both liquids from their radial units and mix them in a measuring cuvette facing the transfer port of each unit (Fig. 8.7b). The sample is also held in place by the centrifugal force for as long as the disc is spun. The cuvettes are placed on an external disc which is also spun. The bottom of the system is made of quartz,

as is the zone over each cuvette. The sole optical system used is in a fixed position (Fig. 8.7c) and continously measures the contents of each cuvette passing through it. A screen continuously displays a bar graph showing the increase in absorbance in each of the 30 cells up to a fixed value in each case. A printer provides the result of each measured parameter. Not only are end-point measurements possible, but the system can also be programmed for kinetic enzymatic measurements.

As all 30 samples are, apparently, measured in a simultaneous fashion, the system can be said to effect a parallel multi-determination —hence the generic name 'parallel fast analyser' given by some workers to these instruments, which are even dealt with separately from batch analysers in automatic methods of analysis. However, such a difference is only apparent and, in fact, it is a typically discontinuous process, both because of the sequential measurement (with a single detector) and because there is a manual intermediate operation (the transport of the transfer disc from one module to the other).

### 8.3.2 Batch analysers without final transfer

In these DAAs, all operations —signal measurement included— are carried out in the same vessel. There are a large number of commercially available analysers of this type. A comprehensive description of them is beyond the scope of this book, so only three representative multi-parameter, non-specific examples are commented on here.

It is significant that Technicon, the pioneering and virtually exclusive developers of continuous automatic analysers, have recently devoted their production efforts to batch models. Probably, their RA-1000 model was created to compete with the centrifugal analysers introduced by other firms and which were gradually displacing continuous-flow AutoAnalyzers, widely used until a decade or so.

The RA-1000 is a compact, computer-controlled analyser consisting of three discs (Fig.8.8a):

(a) A central unit or reaction tray consisting of a disc containing 100 disposable optically transparent (7 mm light path) cuvettes around its periphery.

(b) A sample turntable with 30 0.5-mL cups where samples are held.

(c) A reagent tray, with 25-mL cups.

The system uses high-precision Hamilton pipettes to transfer samples (3-20 µL) and reagents (300 µL) to the central reaction tray. Some inert fluid TRAF, the instrument's most significant innovation, is used to avoid carry-over. Each parameter is measured in a single cuvette; thus, if five parameters per sample are to be determined, the instrument capacity will be 20

(100:5) samples per operation (complete turn of the central disc). In fact, the instrument is capable of analysing for up to 40 parameters per sample. The detection system is a single-filter spectrophotometer with two photodetectors allowing the instrument to carry out a self-adjusting prior measurement of the cuvette cleanliness and the reagent quality before the sample is added to make the final measurement. No stirring is needed.
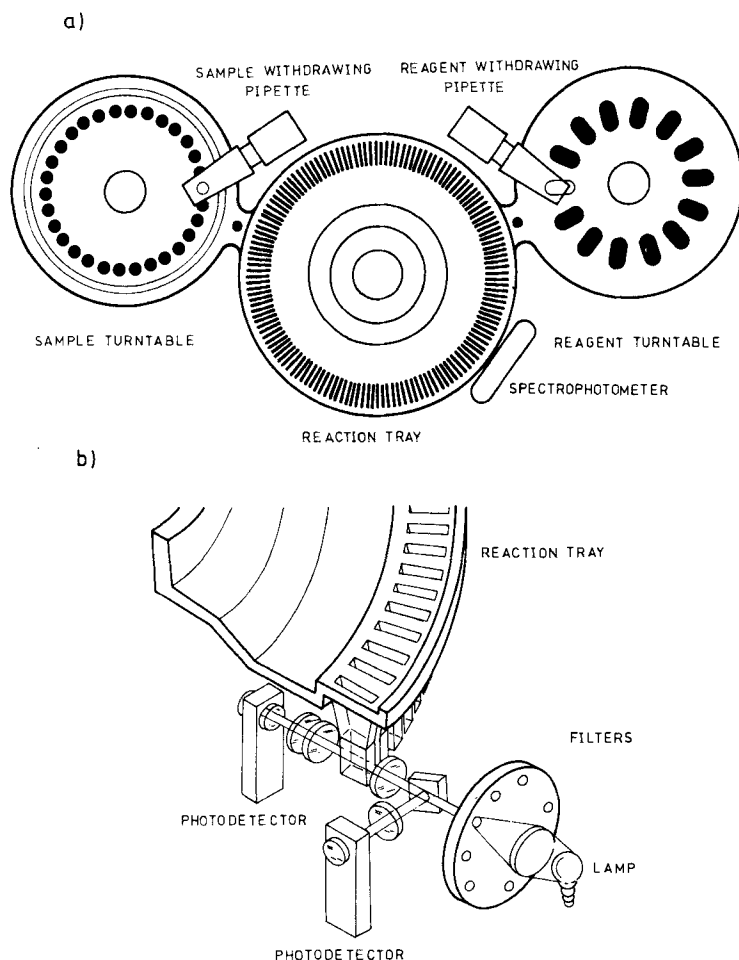


**Fig. 8.8** Scheme of the Technicon RA-100 analyser. (a) Sample, reagent and reaction discs, with two intermediate liquid transfer systems; (b) optical detection system. (Courtesy of Technicon).

The microprocessor exerts control over (a) the number of parameters determined per sample; (b) the type of analysis to be applied in each instance

(end-point, first- or second-order kinetic); (d) the transfer of the sample and reagent pipettes; (e) the circular motion of the three discs; (f) the detector operation (filter selection, blank correction); (g) signal acquisition and processing; (h) result delivery —normally as a print-out—; and (i) urgent delivery of data without alteration to the system.

Even more complex and powerful than the Technicon RA-100 is the Hitachi Model 705, a multi-parameter analyser capable of analysing for up to 19 analytes per sample. As can be seen from Fig. 8.9, it consist of the following elements:

(a) A sample disc with 70 positions, 40 around its periphery for routine samples and 30 (16 for standards, 3 for control sera, 8 for urgent cases, 1 for electrolyte standards and 2 for blanks ◘ 29, no 30) on an inner circle. It is equipped with a sensor controlling the level of the liquid in each vial.

(b) A reaction disc, with 48 non-disposable plastic cuvettes with optically transparent bottoms, located on its periphery. This disc effects a complete turn every 20 s. The cuvettes are immersed in a thermostated heating-cooling bath that controls the temperature to within ±0.1°C.
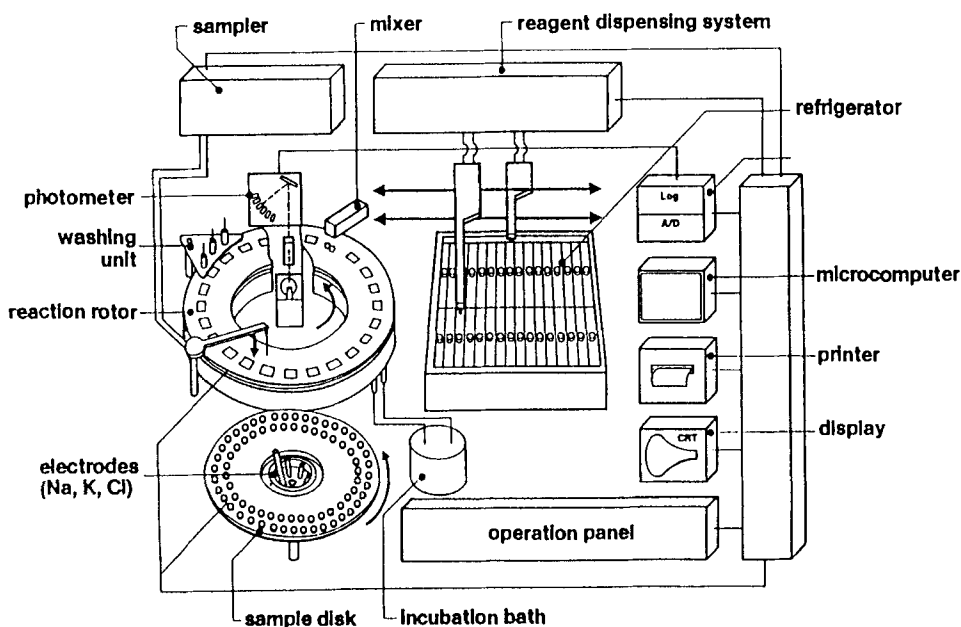


**Fig. 8.9** Essential parts of the Hitachi 705 analyser. (Courtesy of Hitachi).

(c) A flat, thermostated unit containing oblong cuvettes holding the different reagents, diluents, etc.

(d) A liquid transfer system consisting of three moving articulated needles fitted to high-precision syringes, one of which is used to aspirate and pour a small sample volume (5–20 μL) from the sampler to the reaction-measurement cuvette while the other two are employed to dispense variable volumes of reagents/diluents to the reaction cuvettes after withdrawing them from their corresponding reservoirs.

(e) A peculiar photometric sensing system: the light path is vertical rather than horizontal (Fig. 8.9) and it has eleven photodetectors strategically arranged behind the monochromator which allow the absorbance to be measured simultaneously at eleven different wavelengths, thereby enhancing the automatic multi-parameter performance of the analyser. All the cuvettes are monitored every 20 s and the system always carries out a blank determination per cuvette. Naturally, it can be used to implement both end-point and kinetic determinations.

All the above-described operations are controlled by a computer with programs for the fast determination of analytes in urgencies, error checking, throubleshooting, etc. The analyser can be connected on-line to any laboratory microcomputer and features a number of advantages: (a) high sampling frequency; (b) capability of analysing a large number of parameters per sample and the possibility of determining potassium, sodium and chloride potentiometrically by means of their respective ESIs; (c) thermostatic control; (d) a quality photometer rather than a filter spectrophotometer; (e) the possibility of automatically determining in a quantitative manner three clinical parameters (icteric, haemolytic and lipaemic indices) generally determined semiquantitatively by visual inspection.

The FP-9 and FP-01 models manufactured by Labsystems Oy are examples of single-parameter batch analysers automated to a lower degree than the Technicon or Hitachi models described above. Their keypiece is the so-called 'Finnpipette', a multi-pipette combining nine in one and allowing the transfer of nine equal volumes (5 μL–1 mL) in a simultaneous fashion (Fig. 8.10). The nine disposable plastic tips make a block linked to the pipette central barrel. A mechanical system allows them to be detached in a single operation. A block containing nine tubes or vials receives the contents of each sample or reagent transferred manually. This block can be coupled to independent incubation (FP-400) or centrifugation (FP-510) modules. Partial automation is achieved by simultaneous pipetting over nine cylindrical plastic cuvettes (0.2–0.8 mL) with optically transparent bottoms. These cuvettes can be adapted to solid-phase enzymatic immunoassays thanks to supports retaining the solids. The

FP-901 analyser proper is a semi-automatic nine-channel photometer with verti-
cal parallel light paths unlike conventional photometers. It has nine light
sources and as many photodetectors. Measurements are carried out simultaneous-
ly, which makes this a parallel batch system. However, despite its apparent
multi-parameter character, it can only determine one parameter in each batch
of operations. It allows for incubation at 30 or 37°C and for mixing within
each cuvette. Its functioning is controlled by a microprocessor.
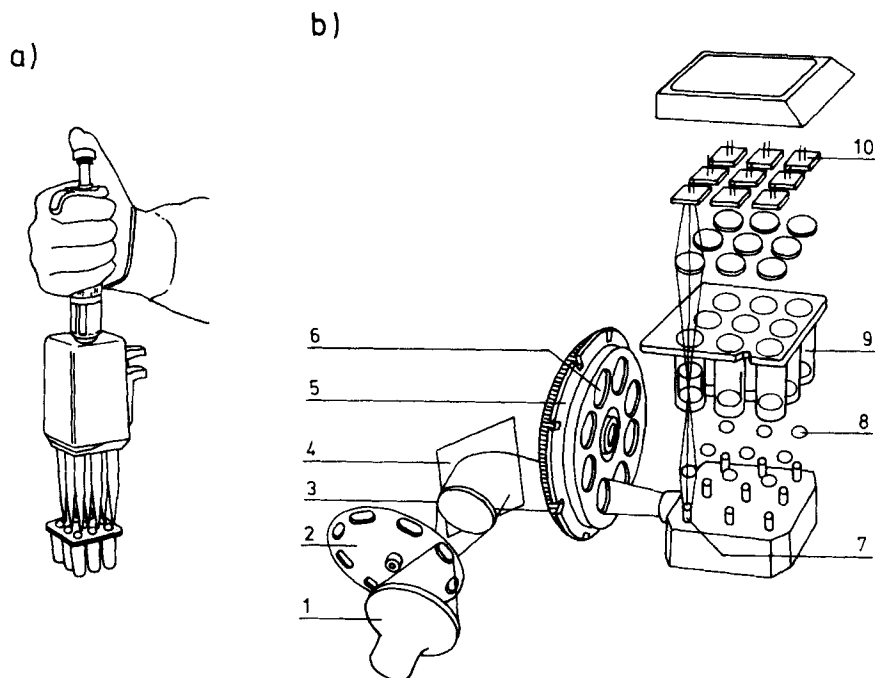


**Fig. 8.10** Scheme of the FP-901 analyser manufactured by Labsystems
Oy. (a) Finnpipette; (b) multiple optical system 1: light source, 2:
chopper, 3: lens, 4: mirror, 5: filter wheel, 6: fibre bundle, 7:
openings, 8: lenses, 9: cuvette block, 10: detectors. (Courtesy of
Labsystems Oy).

## 8.4 BATCH ANALYSERS WITHOUT AUTOMATIC SAMPLING

This section includes those automatic systems in which the sample introduc-
tion unit is differentiated from the main module. They are dealt with here
from the point of view of their flexibility, in which respect they were classi-
fied into general and specific designs (see Chapter 1).

### 8.4.1 General designs

These are analytical configurations capable of determining a large number
of analytes in a variety of samples.

Taking into account the concept of automation given in Chapter 1, many commercially available analytical instruments (optical, thermal, electroanalytical, magnetic) are automated to some degree. As a rule, the last two stages of the analytical process, namely signal measurement and data acquisition and treatment, are performed with no human intervention by a microprocessor coupled on-line with the instrument, which governs part of its functioning and handles data as programmed. This computerization is unnecessary in some instances as it merely solves minor problems or makes for more convenient work. In this respect, routine kinetic determinations have been one of the fields benefiting to a greater extent from computerization: virtually all instruments with microprocessors —even the simplest ones, but particularly spectrophotometers— feature programs for this purpose yielding results expressed as concentrations. On the other hand, computerization plays a major role in some analytical techniques, to the point of owing their existence to the advent of computers. Such is the case with ultra-fast kinetic methodologies (stopped-flow) or Fourier transform spectroscopy (both IR and NMR).

Most of these instruments have sampling accessories —generally coupled on-line— to reduce human intervention in the analytical process. They often also have an independent microprocessor linked to the main one (Fig. 8.11). The aforesaid accessories can have a variety of purposes, namely:

(a) Reducing  human intervention in processing large numbers of samples.

(b) Improving the precision of the analytical determination (e.g. use of a sampler to introduce samples into an electrothermal-vapourization atomic absorption spectrometer).

(c) Facilitating sample pretreatment or part of it. This is the purpose of some of the sampling accessories used in gas chromatography, which look like ordinary samplers but perform automatic non-routine operations in the sampling position. Such is the case with the samplers for air sorption tubes, the contents of which are introduced into the chromatograph by means of a programmed thermal desorption device, or the sampler used for introduction into the chromatograph of volatile substances from liquid or solid samples dealt with in head-space gas chromatography (see Chapter 11).

The incorporation of a sampler into an instrument turns the latter into an analyser. However, the scientific jargon supports the original nomenclature with very few exceptions. Thus, the term 'analyser' is used to describe (1) complex automatic systems where detection is only another aspect among others that are equally important and (2) straightforward specific automatic systems with emphasis on the particular analyte determined rather than on the instrumental technique upon which their operation is based.

Detailed descriptions of the automation of instruments typically used in
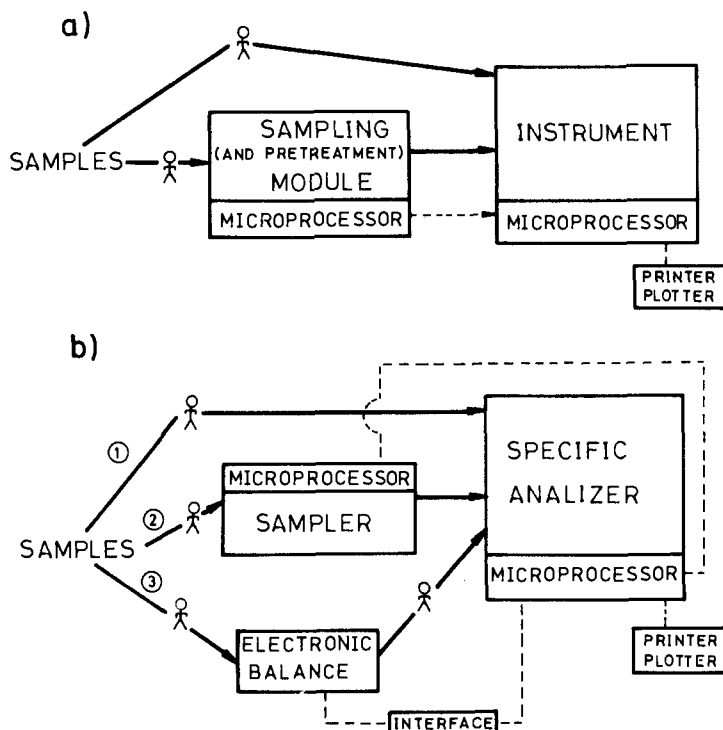
**Fig. 8.11** Commoner configurations of batch automatic systems in which the sampling system is an independent module. (a) Automation of the first stage of the analytical process in optical, electroanalytical, thermal or magnetic instruments. (b) Different ways of incorporating samples into specific analysers.

research and control laboratories, whether in one, several or all of the stages of the analytical process, are given in the chapters devoted to optical (Chapter 10), electroanalytical (Chapter 11) and chromatographic (Chapter 12) techniques. In most of these instances, samples are introduced sequentially, so they preserve their integrity. Hence these can be roughly considered batch analysers. Nevertheless, the conduits along which samples are transported in spectrophotometers with aspiration and in chromatographic processes are shared by all of them, so that these are closer to the concept of continuous analysers (CFA). In fact, the likeness and differences between FIA and HPLC have been discussed by some authors.

### 8.4.2 Specific designs

These are analysers conceived for the determination of one analyte or a few in a given type of sample. There are configurations automated to different degrees, with or without a microprocessor, the commonest of which are shown
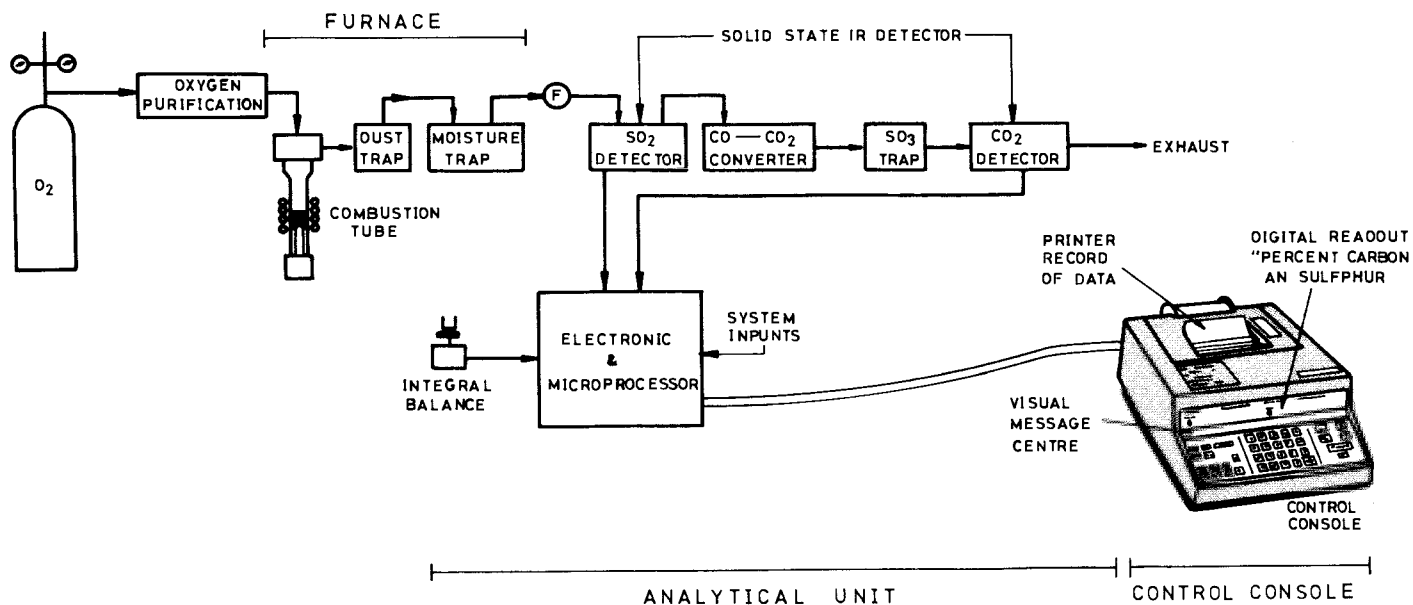
**Fig. 8.12** Main parts of the CS-244 specific analyser manufactured by LECO for the simultaneous determination of carbon and sulphur in inorganic materials. (Courtesy of LECO).

schematically in Fig. 8.11b. The simplest alternative (b.1) involves manual introduction of the samples —previously quantized if needed. The second possibility (b.2) involves the use of a conventional sampler on which the samples are placed manually to be subsequently transferred to the instrument without human intervention. The samples are normally liquid and the transfer is usually accompanied by their quantitation. Configuration b.3 is common with solid samples, which are weighed manually and introduced into the analyser. In order to reduce human intervention in this stage, the analyser microprocessor receives data from the electronic balance on which calculation of the analyte concentration is based. Nevertheless, in the MAC–400, described below, the turntable containing the samples is part of the instrument.

Caution should be applied at this point not to confuse this type of analyser with the variety of automatic modules available for sample preparation which offer no analytical information themselves.

Specific designs have been conceived to resolve special problems in various fields (clinical and metallurgical chemistry, pollution, nutrition, etc.). In fact, they are instruments covered by a denomination referring to the analyte and type of sample for which they are intended. They are particularly suitable for carrying out a more or less large number of crucial determinations in the fields mentioned above. They are characterized by their simple operation (tuning, calibration, sample introduction, delivery of results) insofar as they are intended for unskilled staff —so much so that few control laboratories know their operational foundation (i.e. the particular determinative technique being applied).

There are an overwhelming number and variety of commercially available specific designs. As a comprehensive description is beyond the scope of this book, only some significant examples are discussed. Other analysers belonging to this group are commented on the Chapters 14 and 15, devoted to the automatization of clinical and environmental pollution monitoring.

The CS–244 Simultaneous Carbon/Sulfur Determinator manufactured by LECO is a specific microprocessor-controlled analyser developed for the determination of these two elements in metals, ferrous and non-ferrous alloys and various inorganic materials. As can be seen from Fig. 8.12, it consists of three essential elements:

(a) The *analyser module* proper, which yields the analytical signals. It is governed by the microprocessor and incorporates two determinative modules. One contains the plate of an integral electronic balance where the crucible is placed together with the accelerator; once automatically tared, the sample is added and the weight transmitted to the microprocessor. The other is a manifold carrying oxygen, various clean-up units (dust, moisture and $SO_3$ traps),

flow-rate regulators and two solid-state continuous IR detectors for the detection of $SO_2$ and $CO_2$, in addition to a catalytic bed for conversion of CO to $CO_2$.

(b) The *induction furnace* (18 MHz, 3600 V), an independent module (LECO HF-100). Once the sample has been weighed in the crucible, it is transferred manually to this unit and the cycle is started. The sample is burnt in an oxygen atmosphere, and the gases generated are passed on to the analyser module. High combustion temperatures are microprocessor-controlled for more consistent burns and the result is improved reproducibility, particularly when analysing difficult-to-burn materials  such as nitrides, cast iron or refractory metals. This module also features automatic dust ejection after each determination and semi-automatic cleaning.

(c) A *control console*, connected to the microprocessor. Its keyboard allows the control of most of the functions of the analyser module. Data treatment is effected in much the same way as in gas chromatographs: it is performed by the microprocessor, which receives the two signals (peak)  and calculates the carbon and sulphur contents on the basis of the initial weighing and delivers them via the digital panel or a printer.
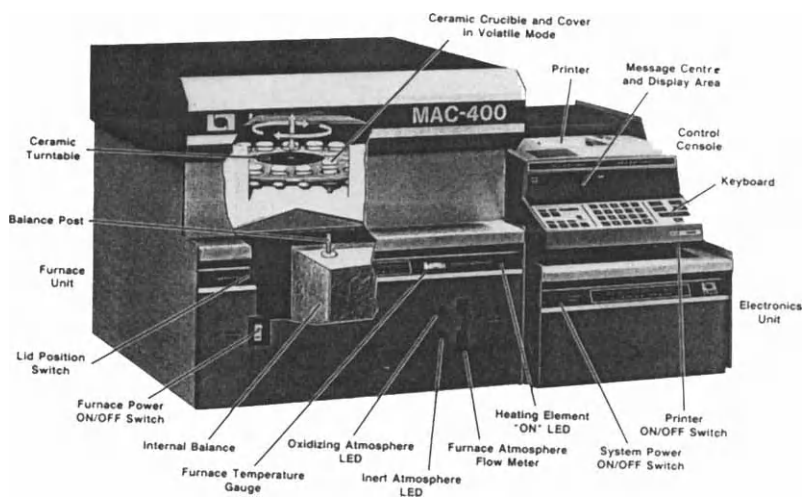


**Fig. 8.13**  LECO MAC-400  Proximate Analyzer, designed for the determination of  moisture, volatile matter, ash  and fixed carbon. (Courtesy of LECO).

The LECO CS-344 has an additional automatic sample loading and unloading feature incorporated into the furnace module. LECO also manufactures models for the individual determination of carbon (IR-212) and sulphur (IR-213) and

even more specifc for the determination of surface carbon on sheet steel and copper tubing (RC–212).

The MAC–400 Proximate Analyzer, also made by LECO (Fig. 8.13), is capable of performing four different determinations (moisture, volatile matter, ash and fixed carbon) in each sample (coal, coke and other organic materials). The instrument operates by monitoring the weight loss of the sample as it is heated in a temperature- and atmosphere ($N_2$ or $O_2$)-controlled environment. It allows the implementation of several analytical alternatives: complete analysis cycle, modified ash mode and any combination of the moisture, volatile matter and ash cycles. The MAC-400 is a microprocessor-based analyser; it allows the most important functions of the analytical furnace unit to be controlled via its console. An electronic unit houses the microprocessor and associated electronics. The furnace unit contains an internal balance system and a turntable with 20 crucibles which rotates samples and lowers them individually on to the balance plate. At the start of the procedure, empty crucibles are placed in the turntable. The furnace top closes to prevent air currents and each crucible is individually weighed. Nineteen samples are then positioned manually into crucibles, which are again individually weighed. The data are collected by the control console and then the analyser proceeds automatically through the selected analysis cycle while continuously rotating and weighing the samples. The apparent changes in the weight of the empty reference crucible are used by the microprocessor to correct the results obtained from the samples themselves.
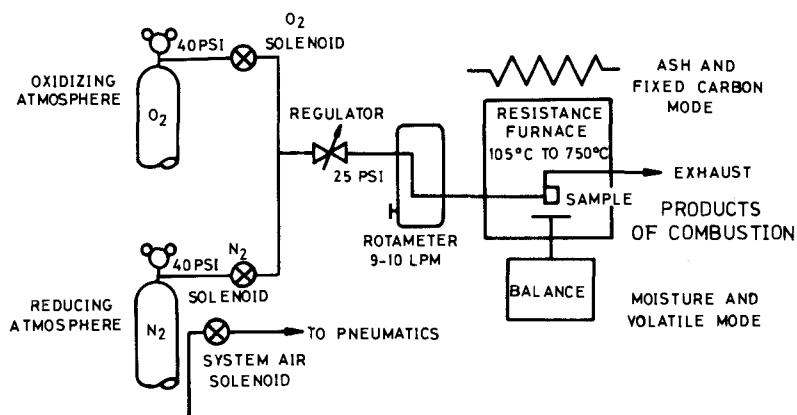


**Fig. 8.14** Gas–flow system of the LECO MAC–400 analyser. (Courtesy of LECO).

A simplified flow diagram of the MAC-400 is shown in Fig. 8.14. During the moisture cycle, the furnace is purged with nitrogen and the temperature is raised to 106°C. Either a constant-weight or a fixed-time mode can be selected for the determination of the moisture content. When this step ends, the analyser emits a bleeping sound to remind the operator to place covers on the crucibles, which are tared again. Then the furnace temperature is raised to 950°C in a nitrogen atmosphere. The weight loss is related to the volatile matter content. After the volatilization cycle is complete, the temperature is lowered to 600°C and the analyser again bleeps, this time to remind the operator to remove the crucible covers. Then, an oxidizing atmosphere is introduced and, after a programmable period of time, the furnace temperature is raised to 750°C, where it remains until the samples again attain constant weight. Any weight loss during this final cycle is attributed to fixed carbon, and any weight remaining is considered to be the ash content. The printer finally delivers the weight percentages corresponding to the determined parameters. The microprocessor and the console can simultaneously control the operation of two MAC-400 analysers, so that 114 samples can be analysed every 8 h.

The high cost and sophistication of large instruments such as NMR and X-ray fluorescence spectrometers make them unaffordable by control laboratories. Thus, some firms have marketed analysers based on the same principles but designed for specific determinations not requiring too high precision, but rapidity and efficiency. Such is the case with the MK-III and MK-4000 analysers manufactured by Newport Instruments, based on NMR measurements of the hydrogen content of solid and liquid samples for the rapid (a few seconds) determination of the fat and moisture content in a large variety of samples. A detailed description of these instruments is given in Chapter 15. LECO also markets several microprocessor-based analysers using X-ray fluorescence measurements for the individual determination of silicon (XR-122), phosphorus (XR-123), manganese (XR-124) and all three (XR-172) in cast iron, steel, ferrous alloys and non-ferrous materials. Sampling, sample treatment and determination require less than 5 min overall and all the analysers offer direct readouts of percentages of Si, P and Mn.

# 9
## Robots in the laboratory

### 9.1 INTRODUCTION

Robotics is currently one of the priority topics [1-3] in research and development strategies in advanced scientific communities, so much so that there are already some organizations, such as the Robot Institute of America and the British Robot Association, specifically devoted to this subject. Nevertheless, the prospects for the use of robots in different fields of application greatly surpass their present achievements.

Insofar as robots are designed to replace human effort partly or completely, their applications can be considered the latest alternative to automatic methods of analysis. Although some workers regard them as a part of batch methods, there are sufficient distinctive elements to treat them as a separate category.

The use of robots in the laboratory is one of the strongest trends in the field of analytical instrumentation. Irrefutable proof of the growing interest in robotic applications is the recent establishement in the USA of commercial firms devoted exclusively to this area. Several other manufacturers have already introduced robots in their most recent ranges. Prestigious scientific journals such as Analytical Chemistry [4] and Science [5] considered robotic systems to be the most outstanding novelty at the Pittsburgh Conference in 1985. In addition, an increasing number of courses, seminars and symposia are being organized on both sides of the Atlantic.

The application of robots in the laboratory is still at an early stage of development. However, it is easy to predict that robots will occupy a prominent place in laboratories in the years to come. Nevertheless, despite the popular connotations behind the word 'robot', technology is not yet in a position to offer inexpensive, intelligent moving machines in the style of the well-known androids of science-fiction films.

There are clear precedents for the use of robots in the laboratory. So far there have been great advances in the development and manufacture of robots for industrial purposes, and these will ultimately facilitate the incorporation of minirobots into the laboratory. On the other hand, the most representa-

tive precursors of today's robots are probably the manipulators developed for handling radioactive or hazardous samples over the past few decades.

The development of robots has obviously relied on the spectacular advances in three scientific areas, namely micromechanics, microelectronics and microcomputer science. No robot can be considered to be a useful piece of equipment if its motion cannot be precisely controlled by a suitable computer, good proof of which is the fact that the National Bureau of Standards has designed a device for controlling the precision of robot movements [6] consisting of a laser interferometer, a servo-controlled tracking mirror, a target mirror mounted on the robot's wrist and a computer governing the whole system.

## 9.2 CHARACTERISTICS OF ROBOTS

Robots are sophisticated machines whose movements resemble those of a human being, aimed to perform a well-defined operational sequence. According to the Robot Institute of America, a robot is "a reprogrammable, multifunctional manipulator capable of moving a variety of tools and parts, through a variable, preprogrammed task." It is important to make a clear distinction between robots and other types of automatic mechanical systems. In fact, robots have two distinct features in this regard, namely programming —and even reprogramming and self-programming— capability and flexibility for adaptation to different tasks or situations. The word 'robot', on account of its novel implications, is sometimes misused in the literature [7] to describe other automatic devices in an attempt to enhance their significance.

### 9.2.1 Classification of robots

Robots used for industrial or laboratory purposes can be broadly classified according to their interaction with the outside world and their programming capability into two general categories: sensorless and sensor-bearing.

*Sensorless robots* lack the possibility of communicating with their environment and can be further classified into two basic types: computer-controlled or 'first-generation' robots, which can be programmed by means of a computer system, and training robots, which merely repeat a sequence of moves under the direct and continuous control of a human operator.

*Sensor-bearing robots* can communicate with the outside world via different types of sensor (optical, acoustic, tactile, etc); they can also be externally programmed and reprogrammed and take decisions in real time (i.e. they can program themselves with the aid of computers accommodating suitable feedback mechanisms). These 'second-generation' or 'intelligent' robots are endowed with the so-called 'artificial intelligence', which is still at an early stage of development.

### 9.2.2 Robot components

Despite the great formal differences between the various possible configurations of robotic systems possible, these can be said to consist of four basic common elements (Table 9.1), three of which (manipulator, control system and power supply) are indispensable, whereas the fourth (sensor) is characteristic of second-generation robots.

**TABLE 9.1**

Essential components and features of a robot

| Manipulator | Essential elements | Body |
| | | Arm |
| | | Gripper (hand) |
| | Spatial geometry | Cartesian |
| | | Cylindrical |
| | | Spherical |
| | | Revolute |
| Power supply | Hydraulic | |
| | Pneumatic | |
| | Acoustic | |
| Controller | Point-to-point programming | |
| | Software | |
| Sensing system | Location | On the robot |
| | | Off the robot |
| | Types | Optical |
| | | Acoustic |
| | | Tactile |

The manipulator constitutes the mechanical system of the robot and is composed of two essential elements: various parts, linked via suitable joints (kinematic pairs), which are responsible for the robot's movements (translation and rotation), and gripping devices which mimic the actions of the human

hand (grasping, orientating, moving and releasing the manipulated pieces). Overall, a robot's mechanical system consists of a body, an arm and a hand (gripper), all of which are moving.

Depending on the coordinate systen characterizing the robot's motion and defining the spatial position of its hand at a given moment, robotic systems can be classified into four spatial categories [8], namely:

*Cartesian.* Robots belonging to this group can only perform translational movements, which are defined in a three-dimensional coordinate $(x,y,z)$ system.

*Cylindrical.* Here the hand's position is defined in cylindrical coordinates $(x, z$ and the angle $\theta)$. The robot is therefore capable of performing both translational and rotational movements (Fig. 9.1a).

*Spherical.* The hand's position is given in spherical coordinates $(x$ and angles $\theta$ and $\Upsilon)$ and its movements are basically rotational.

*Revolute.* In this case the robot's movements are exclusively rotational (Fig. 9.1b). The essential difference from the other three is that, in addition to the rotatory motion of the base, the arm is jointed in the human style and includes a shoulder, an elbow and a wrist, all of which can perform angular movements.

A                                                          B
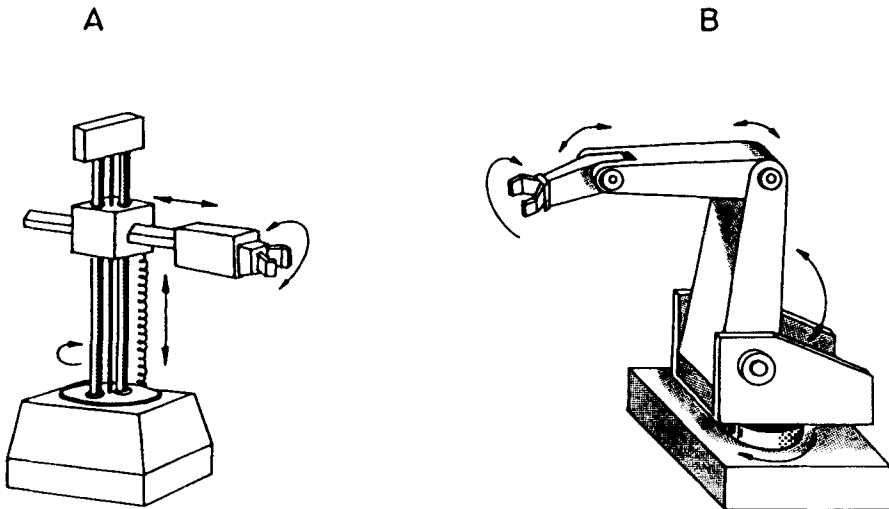


**Fig. 9.1** Commonest configurations of laboratory robots. (a) Cylindrical. (b) Revolute.

There are several interesting papers [4,12] in which the advantages and disadvantages of each type of robotic configuration are discussed.

The gripper is essential to a robot. It emulates a human hand, although it is still a long way from achieving the same degree of freedom. The gripper can

be either fixed or interchangeable for greater versatility. It is usually manu-
factured in one of three different configurations:

(a) With non-flexible fingers capable of angular or parallel motion only.
This is the simplest alternative.

(b) With interrelated fingers which allow the pressure applied on the ob-
ject to be regulated according to its fragility.

(c) With multi-jointed fingers, which in turn can be retroflexive —very
similar to their human counterparts—, close around the object or form two
parallel sets facing each other.

The incorporation of tactile sensors into grippers allows for increased re-
liability and easier programming.

The power supply provides the energy needed to move the different parts of
the manipulator. It is of great relevance as it determines the robot's speed,
precision and resolution. Not only the type of power source used, but also its
location in the system is important. Hydraulic sources are essential when
dealing with heavy objects, whereas pneumatic systems are employed when quick
movements are required. Robots used in laboratory applications are usually
driven by electric energy. An electric servo motor or stepping motor is usual-
ly employed depending on whether moderate or light loads are to be handled.

The robot's controller is usually a computer or microprocessor which pro-
grams its operation and sends it the corresponding drive signal. The robot can
be controlled in two different fashions:

(a) By point-to-point programming, which involves driving the robot manu-
ally and directing every stage of the operational sequence from a teach
pendant box where the robot's movements are suitable reflected. Robots thus
controlled are similar to radioactive sample manipulators and are of little
practical interest; in fact, their chief use is as substitutes for humans in
clean chambers (e.g. in trace analysis).

(b) By use of sowftware allowing the operational sequence to be programmed
via the keyboard of an ordinary computer. According to Kool and Michotte [9],
four different levels can be considered in programming a robot's motion: (1)
The *joint level* (the lowest), where the programmer has to decide by what
amount each of the robot arm joints has to rotate (or translate) to achieve a
desired position through a teach pendant box. (2) The *coordinate level*, at
which the programmer can specify the coordinates of the point to be reached
and the orientation to be realized. Most software available nowadays works up
to this basic level and was developed from known languages such as BASIC,
Pascal or Algol. (3) The *object level*, which allows the user to issue instruc-
tions of the type "put the object A on object B." The initial position, orient-
ation and size of the objects are retrieved from stored data and trajectories

and new positions are automatically calculated. This level requires 'computer-aided design and planning.' (4) The *task-programming level*, at which the programmer asks the flexible automated analysis system to carry out a given analysis or to determine or synthesize selected products. It is an expert system affording complete flexibility.

In addition to controlling the robot's motion, the computer performs a variety of tasks such as the control of the apparatus and intruments used and the collection, processing and delivery of generated data.

The robot's *sensing system* allows it to be reprogrammed for different situations (e.g. when objects of different weights, sizes, fragility, etc. are to be handled). The robot feels, sees, hears and responds to these stimuli by changing its movements. Therefore, sensor-bearing robots are more flexible than their sensorless counterparts insofar as they are prepared to face unexpected situations. The sensing mechanism can be placed in two basic parts of the robot, namely the manipulator (usually on the gripper) or a point at which the gripper has to be stopped and manipulated.

Robots use three basic types of sensor, depending on the nature of the signal utilized for communication: optical, acoustic and tactile [10].

The simplest optical systems employed by robots consist of LED-photodetector pairs mounted on the hand. The presence of the object to be manipulated is detected when this interrupts a light beam, the finger movements being activated as a result. Although a conventional TV camera may serve this purpose, linear or square diode arrays (with 250–1000 and 250x250 elements, respectively) radiated by a single source afford greater information.

Acoustic sensors have been less frequently used on robots. They are generally based on the use of a pulsating ultrasound source (*ca.* 40 kHz) and the accurate measurement of the time interval between transmission and reception of the pulse.

The most straightforward type of tactile sensor used consists of a contact switch which provides binary high–low information. Other types of mechanism such as strain gauges and carbon fibre materials change their electric resistance under stress, whereas piezoelectric materials generate an electric charge when subjected to mechanical strain.

Hirschfeld [11] presented an interesting discussion on  the incorporation of sensing systems into robots commonly used in laboratory applications. Among these and deserving of special consideration is a simple photometer which, by use of different filters, can control a series of interesting operations such as precipitation, decantation, boiling, dissolution and extraction. The workpiece (generally a test tube) crosses the light path which monitors the different zones. Figure 9.2 illustrates the operation control of a precipitation

process in a test tube by use of a horizontal light path filter in a conventional photometer. It also includes the response of the sensor as a function of the tube height.
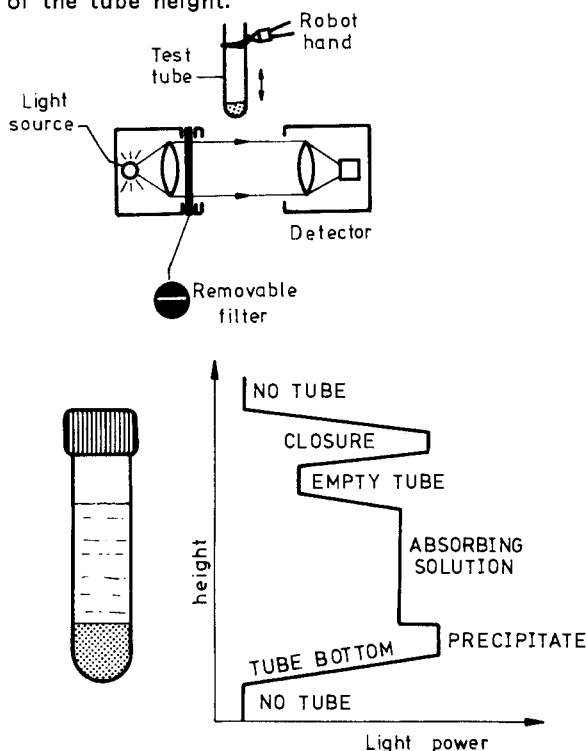




**Fig. 9.2** Use of a photometer as a sensor in a laboratory process controller. A tube is introduced into the ligh path of the photometer, which accommodates a horizontal filter. (Reproduced from [11] with permission of Elsevier).

Commercially available laboratory robots (e.g. Perkin Elmer) have been conceived for use in corrosive environments. They have hard-wearing spare parts and a special system delivering an inert gas stream which flows continuously over the robot's micromotors and electronic circuits to prevent them from coming into contact with the aggressive atmosphere in which they usually act.

A major limitation of laboratory minirobots with respect to human operators is their action range (usually restricted to within 50–70 cm); in other words, they have no feet to move along a workbench, which in turn limits the number of peripherals that can be fitted to them. This shortcoming can be overcome by mounting the robot on rails along which it is moved, also under the control of a computer.

### 9.3 LABORATORY ROBOTIZATION

Every analytical process is the sum of three fundamental operations: preliminary manipulation, measurement and transducing of the analytical signal, and data compilation and processing. It is the last two stages which have experienced the greatest extent of automation in the past 20 years or so. The incorporation of a micropocessor for the automation of instrumental operations is becoming commonplace in most commercial instruments —even in the less expensive models. Data acquisition and treatment are readily affordable at present and is carried out with the aid of built-in microcomputers or by use of ancillary modules (personal microcomputers, electronic integrators) coupled on-line with the instrument in question.

It is the first stage of laboratory analytical methodologies which poses the greatest problems for automation (see Chapter 2). Operations such as weighing, dissolution, grinding and centrifugation are difficult to incorporate on-line in automatic analysers (whether batch or continuous). It is therefore here that robotic systems cover a field inaccessible to the remainder of automatic methodologies.

This first stage is as important as or even more so than the other two inasmuch as (a) they are a major source of a variety of errors —some of them so large as to decisively influence the final result—, (b) they are time-consuming, (c) they are complex and expensive and (d) the system is subject to human contamination, which is a high risk in trace analysis, for instance.

Laboratory robotization is chiefly aimed to the first few stages of the analytical process —hence its relevance to analytical procedures involving long, multi-stage preliminary operations.

There are two basic options for incorporation of robots into laboratory work:

(a) The robot can be an independent module performing one or several specific tasks within the system framework. The microprocessor is exclusively devoted to programming and control of the robot and additional computers are required if other operations of the overall process are to be controlled.

(b) Alternatively, the robot can be an integral part of the general work system, so that every operation —from sampling to delivery of results— is controlled by a single computer. This is the more reasonable, economical and frequently used option and is represented by the so-called 'robostation', a sort of analytical black-box [13].

Figure 9.3 shows the general scheme of a robotic workstation consisting of four essential elements: the robot; the micromputer (which can be either dedicated or indeed any commercially available machine); apparatus such as liquid dispensers, mixers, extractors and centrifuges performing a variety of tasks;

instruments such as balances, pH-meters, spectrophotometers, fluorimeters, atomic absorption spectrometers and gas or liquid chromatographs, aimed at providing analytical information. Several other ancillary elements such as racks (intended to hold tubes, Erlenmeyer flasks, sorption columns or burette tips) and waste bins make up the robotic system.
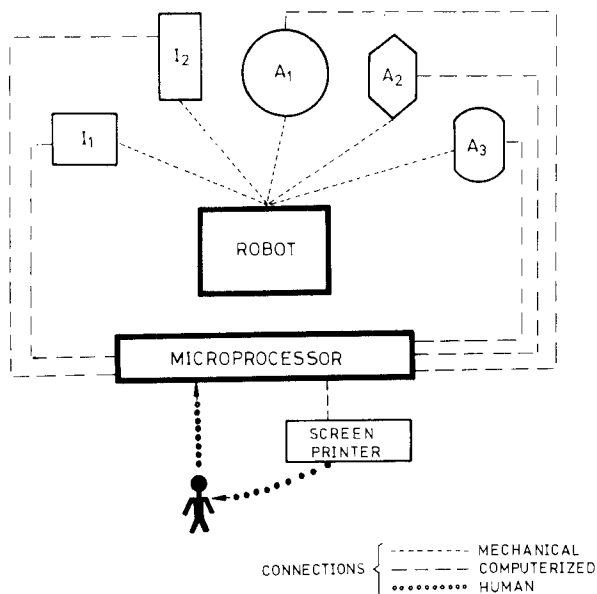


**Fig. 9.3** Scheme of a robotic workstation. ($I_1$, $I_2$ and $I_3$ denote instruments; $A_1$, $A_2$ and $A_3$ represent apparatus.

There are three types of connection in a robotic station: (a) human, between the computer and the operator; (b) computerized, between the microprocessor and the robot and the different apparatus and instruments; and (c) mechanical, between the robot and its environment.

The general functions allocated to the microprocessor [14] are thus: (a) enabling the operator to plan the experiments via the keyboard entry; (b) controlling the robot's motion; (c) effecting communication between robot, peripherals and instruments; and (d) data acquisition and processing.

The connection or linkage between the microprocessor and the different instruments or apparatus making up the system can be of either of these two types, unidirectional or bidirectional.

(a) *Unidirectional.* The instruments send their analytical signals to the

microprocessor, which in turn controls the switching of the different appara-
tuses.

INSTRUMENTS → MICROPROCESSOR → APPARATUS

(b) *Bidirectional.* The microprocessor not only collects the signals from
the instruments, but also takes part in their operation (e.g. in selecting a
suitable wavelength for a spectrophotometer or programming the temperature of
a chromatographic furnace), usually via another microprocessor coupled to it.
The apparatus can also be bidirectionally linked to the microprocessor, par-
ticularly if they accommodate sensing elements involved in one or several
steps of the process (e.g. in locating the centrifuge tubes).

INSTRUMENTS ⇌ MICROPROCESSOR ⇌ APPARATUS

## 9.4 UNIT OPERATIONS
This section deals with the most relevant operations that a robot can per-
form as part of a workstation. In some cases, a given operation is repeated
along the operational sequence.

### 9.4.1 Weighing
The automation of the weighing operation is virtually indispensable, par-
ticularly with solid samples, although it also simplifies to a great extent
the weighing of liquids, which is difficult to automate by other methodol-
ogies.

The  weight of sample taken, measured within a certain limit of accuracy,
is sent to the microprocessor, which stores it as a basis for the final cal-
culations. The typical operational cycle involves the following stages: (1)
taring of the sample flask; (2) placement of the required amount of sample in
the flask; (3) reweighing of the flask; (4) removal of the flask from the bal-
ance; and (5) weighing of the balance pan in case some spillage has occurred
in the previous operations. All five stages are controlled by the micropro-
cessor which, after some computations, determines the sample weight and stores
it for future calculations.

### 9.4.2 Addition of liquids
Robots usually include a special addition unit for dispensing liquids such
as solvents, diluents and reagent solutions to the sample flask. Such a unit
normally consists of one or several systems made up of a solution reservoir, a
high-precision syringe and a T-shaped valve that can be switched between two

positions: filling of the syringe and unloading of the liquid in the prese-
lected  position (Fig. 9.4). The syringes are driven pneumatically or elec-
trically. The liquids are poured into the collecting tubes thorough a fixed
syringe or interchangeable tips —replaced by the robot itself— in order to
avoid carry-over.
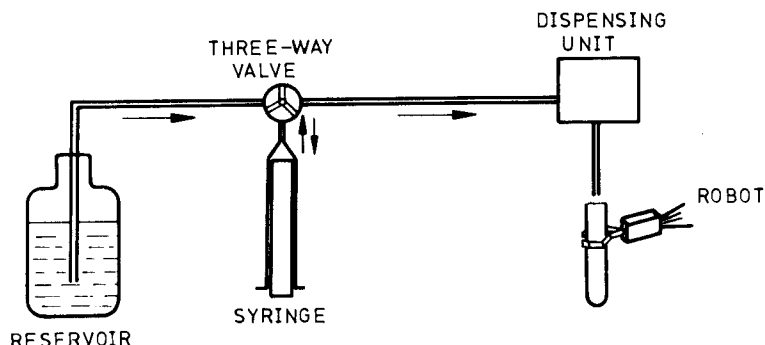


**Fig. 9.4** Liquid dispensing unit with a fixed addition point.

### 9.4.3 Transfer of liquids

As shown in Fig. 9.5, the transfer of liquids can be accomplished in two
ways: by pouring from tubes or flasks, or with the aid of an aspiration-injec-
tion syringe fitted to a disposable tip which is grasped by the robot and im-
mersed in the solution, from which it takes an aliquot that is subsequently
unloaded into the collector.

### 9.4.4 Grasping of tubes

This is a common operation in workstations. Tubes are taken from and
placed in racks after determining the exact position for collection and deliv-
ery. This is accomplished by means of the computer, which takes three corners
of the rack as reference. Any subsequent location operation is based on the
previously fixed references.

### 9.4.5 Stirring

This operation is performed at a definite location within the workstation
and requires careful programming of the period during which the flask con-
cerned will be stirred.

Robotic workstations employ there basic types of stirring unit: *vortex*, which is suitable for tubes only; *magnetic*, particularly suited to beakers and Erlenmeyer flasks; and *linear*, capable of handling several vessels at a time and particularly useful for slow digestions.
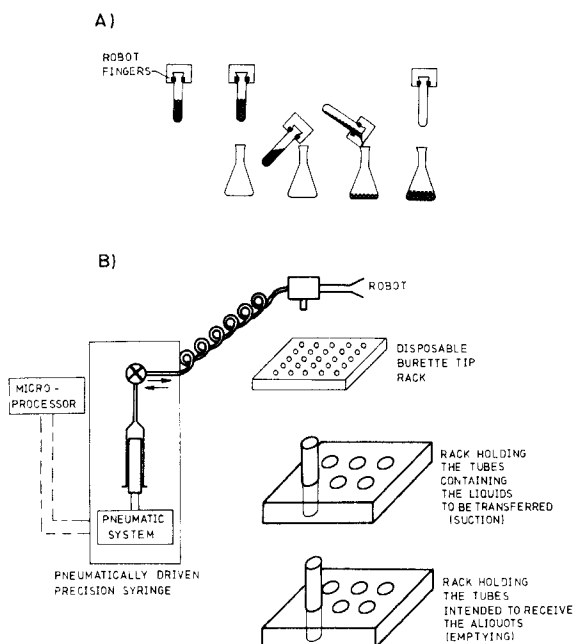


**Fig. 9.5** Liquid transfer systems used in robotic workstations. (A) By pouring.  (B) By pipetting with a high-precision syringe. The transfer can  be effected either by taking both vessels to a fixed location (as in Fig. 9.4) or by means of a disposable moving pipette.

### 9.4.6 Heating

Heating units are usually required to speed certain physico-chemical processes and normally consist of thermostated baths (either water baths or dry-type units such as the Peltier system) in which the robot places the tubes containing the reacting mixture and where these are kept for a preselected period.

### 9.4.7 Centrifugation

Like weighing, this operation, so commonly needed as a vital step in sample pretreatment, is difficult to automate by other methodologies.

The most important part of centrifuges used in robotic stations is prob-

ably a sensor external to the robot, mounted in a fixed position on the centri-fuge and consisting of a straightforward LED-photodetector system. The centri-fuge rotor has an indexing tab on its periphery which, on passing through the sensor, interrupts the light path and generates a signal. This is how the se-quential situation of the tubes within the centrifuge is determined. Before introducing a tube, the tab is aligned with the optical sensor. Then the robot places the tube in the centrifuge and rotates the rotor by the amount needed to introduce a second sample, and so forth. Once all the samples have been placed in the centrifuge, the latter is spun for a preselected time during which the microprocessor is completely insensitive to the signal generated by the optical sensor. On stopping the rotor, the situation of the tubes can only be determined accurately by having the robot rotate the rotor until the index-ing tab is aligned with the optical sensor.

### 9.4.8  Extraction

This operation can be of either of two types:

(a) *Solid-liquid extraction.* This is the more simple of the two. To a given amount of sample held in a vessel is added a fixed volume of liquid(s). After suitable stirring —and heating if necessary—, an aliquot of the super-natant is withdrawn and delivered to another vessel. This operation can be re-peated as many times as required and the extracts gathered in a single vessel.

(b) *Liquid-liquid extraction.* This operation, rather more complex than the previous one on account of the difficulty involved in phase separation, is performed in much the same way as in manual procedures. Thus, once both phases have been added, the tube or flask is shaken (stoppered) for a preselected time. After decanting, phase separation is accomplished either by immersing a pipette tip fitted to an aspiration-pouring system deep enough to take the heavier or lighter phase, or by using a decatation funnel as collector (in this case, the heavier phase is separated from the lighter phase with the aid of a stopcock or solenoid valve).

### 9.4.9 Placement in the measuring instrument

The sample or the reaction mixture can be placed on the measuring instru-ment in two ways (see Fig. 9.6):

(a) Directly, through a conventional sampler. The robot places the  sample vials —usually capped elsewhere in the station if used for chromatographic purposes— on the turntable. Alternatively, the robot can fill the cups al-ready situated on the turntable with aliquots of sample taken with the aid of a pipette tip fitted to a loading-unloading system.

(b) Indirectly, by placing the sample tube in a given position (measure-

ment point). The are two alternatives. One involves taking the sample to the instrument by means of an aspirating tube (the robot can either take the sample tube to a fixed location for aspiration or bring the aspirating tube to the sample rack and withdraw the sample). This is the typical procedure followed for introduction of samples in atomic spectrometric techniques, filling of the injection valve loop in chromatographic methods and in (un)segmented continuous analysers, or loading of a flow-cell already positioned in the light path of a photometer or fluorimeter. The other possibility entails the use of a sensing probe (off the instrument), which is introduced by the robot into the sample vial. This is the usual approach in using potentiometric or voltammetric electrodes or photometric probes.
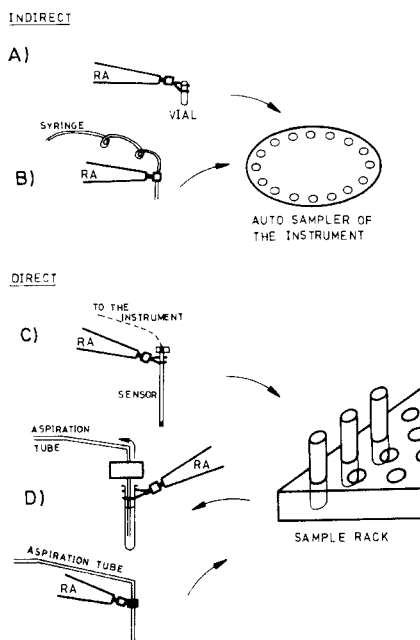


**Fig. 9.6** Different mechanisms for placement of the final analytical solution in the measuring instrument. Indirectly, via the instrument autosampler, which can receive either a vial (A) or a sample aliquot to be held in cups (B). Directly, by means of a sensor (C) or by aspiration (D) at a fixed point where the tube is taken or with the aid of a moving aspiration tube, which is inserted by the robot arm (RA) in each tube in the rack in turn.

## 9.5 SOME CHARACTERISTIC EXAMPLES

The four applications commented on below have been selected because they

are representative of the potential of laboratory robotization. The first two are described in greater detail than the others.

### 9.5.1 Colorimetric determination of formaldehyde in water

The procedure concerned is based on the formation of an intensely pink dye between the analyte, p-rosaniline and sodium hydrogen sulphite. No sample pretreatment is required.
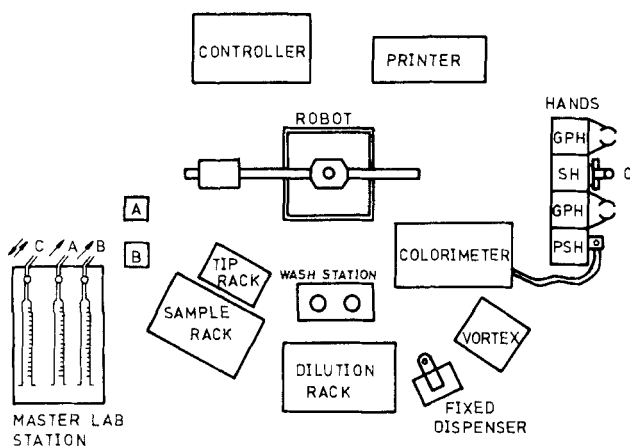


**Fig. 9.7**  Robot station for colorimetric determination of formaldehyde in water. (Courtesy of Zymark).

The workstation used for this determination [15] is depicted schematically in Fig. 9.7 and consists of the following components:

(a) A microprocessor interfaced to printer.

(b) A Zymark robot working in a cylindrical coordinate system and using three types of hand: two grippers for grasping tubes (GPH), one with a tip fitted to a syringe (SH) and a third one accommodating a photometric probe (PSH) linked to a conventional photometer via an optical fibre.

(c) A unit for loading and unloading of liquids (master lab station) consisting of three syringes fitted to three-way valves —two of the ways (A, B) are used to unload reagents while the other (C) acts as a pipette, withdrawing and delivering liquids.

(d) A dispensing unit for addition of a fixed volume of liquid.

(e) Several racks holding sample tubes, disposable pipette tips and clean tubes for dilution and reaction development.

(f) A unit devoted to washing the photometric probe.

(g) A vortex stirring station.

(h) A conventional photometer.

The operations carried out by the workstation are as follows:

(1) The operator places the water samples in their correct order in the sample rack.

(2) The operator, with the aid of a hand fitted to a syringe (SH), takes an interchangeable tip, withdraws an aliquot of sample from a tube and transfers it to a clean tube in the dilution/reaction rack.

(3) The robot replaces its hand by a gripper (GPH), with which it takes the tube containing the sample aliquot and takes it to the dilution unit, where 10 mL of distilled water are added. Next, the tube is taken to the master lab station, where it is first positioned on the tip fitted to the syringe, which adds 1 mL of p-rosaniline, and then to the fixed tip connected to the other syringe, which adds 1 mL of the sodium sulphate solution. Then the tube is kept in the vortex stirrer for 30 s and subsequently returned to the starting point, where it is allowed to stand for 1 h.

(4) The robot switches hands again and takes the photometric probe (PSH) and dips it into the tube containing the reaction mixture. Once the measurement has been carried out, the probe is placed in the wash station, in which it is first immersed in a renewable water bath and then in a drying chamber through which a nitrogen stream is passed. The absorbance reading (signal) is collected by the microprocessor, which treats data on the basis of previous calibrations performed with standard solutions of formaldehyde. The results corresponding to the series of samples are delivered through the printer. If the absorbance measured falls out of the linear range of the calibration graph, the robot repeats the operational sequence, either taking a larger aliquot or diluting the starting solution to a suitable extent as required. In either case, the microprocessor takes into account these operations in calculating the formaldehyde concentration.

During the time each sample is allowed to stand before being measured, the robot treats other samples in sequence.

This example is illustrative of a not very complex determination in which the use of a robot is recommended when the sampling rate required is not very high. The water samples are placed in the rack as they are received in the laboratory. When a reasonable number of them has been gathered, the station is set up to perform the above-described operational sequence. In the meantime, the robot can be used for other applications.

### 9.5.2 Determination of a toxic agent in feed by gas chromatography

This is a representative example [16] of those determinations in wich sample pretreatment is essential to the analytical procedure and where robotiza-
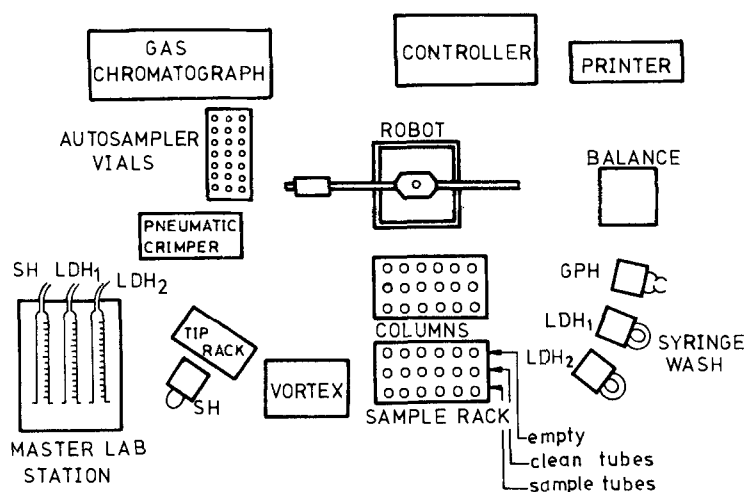
**Fig. 9.8** Robot station for determination of a toxic agent in feed by gas chromatography with a prior liquid-liquid extraction. (Courtesy of Zymark).

tion plays an important role as a means of automation —in fact, this type of determination is difficult to implement by any other automatic methodology.

Figure 9.8 depicts the different elements making up the robotic work-station used for this purpose, namely:

(a) The robot and the microprocessor (interfaced to a suitable printer).

(b) Four different hands —one of which (LDH₁) accommodates a wash unit while the other (LDH₂) has a device for holding/releasing columns, and a multi-functional hand (gripper, GPH).

(c) The master lab station (MLS), which handles solvents with the aid of three syringes fitted to SH, LDH and LDH₂.

(d) Four racks containing the tubes holding the solid samples, disposable Florisil columns (each with a clean collecting tube), disposable pipette tips and unsealed capped chromatographic vials.

(e) A vortex mixer.

(f) An automatic pneumatic vial capping unit.

(g) An analytical balance.

(h) A gas chromatograph with an autosampler.

(i) Three verification systems (an optical sensor surrounding the vial cap-ping station, a push-button switch for pipette tips and a magnetic reed switch at the parking station).

The operational sequence performed by this workstation involves a number of steps, namely:

(1) Ten preweighed tubes each containing an aliquot of feed sample are placed in the sample rack and their weights are stored by the computer.

(2) The robot grasps a tube with its gripper (GPH) and takes it to the vortex mixer.

(3) The robot switches hands. By means of $LDH_1$, connected to the master lab station, it adds 10 mL of toluene to the solid sample.

(4) The tube is shaken for 2 min and then allowed to settle for a further 1 min.

(5) With the same hand ($LDH_1$), the robot pipettess 7.5 mL of the supernatant extract and pours it into a clean tube in the rack.

(6) Two identical solid–liquid extraction operations are carried out simultaneously, but adding only 4.5 mL of toluene each time.

(7) The robot switches hands; it replaces $LDH_1$ —which is washed in its proper place— with GPH.

(8) The robot takes the tube containing the treated sample and returns it to the rack; then it grasps the tube holding the extracts and takes it to the vortex, where it is agitated until homogenization (20 s), and then back to the rack.

(9) There is another switch of hands. With the SH on, the robot takes a disposable pipette tip and aspirates 0.5 mL of the extract, which is unloaded into a Florisil column.

(10) The robot releases the pipette tip and switches hands. With the aid of $LDH_2$ it places the column and its collecting tube on the balance —the pan of which is prepared to accommodate the tube—, which is then tared. The sample is eluted with toluene into the tube. The addition operation, which is facilitated by passing nitrogen through the column, is halted on reaching a given weight. The microprocessor stores the final weight.

(11) The robot discards the used column and replaces $LDH_2$ with GPH, with which it takes the tube from the balance to the vortex mixer and then to the rack. Then it removes the cap from a vial and holds it.

(12) The robot switches hands and with SH takes a pipette tip and transfers (in conjunction with the MLS) 1.5 mL of the clean extract to the uncapped vial.

(13) The robot now replaces SH with GPH, caps the vial and takes it to the pneumatic crimper, where it is sealed. Then the vial is placed on the auto-sampler of the gas chromatograph.

The data supplied by the instrument are transmitted to the microprocessor, which, on the basis of the initial sample weight introduced by the operator, provides the content or concentration of the toxic agent in the feed sample.

The use of the robot in this determination involves two drawbacks, namely

decreased sampling frequency and recovery, which are outweighed by two significant advantages, *viz.* reduced human intervention and around-the-clock working.

### 9.5.3 Liquid chromatographic determination of a contraceptive in pharmaceutical tablets

The use of robotic systems is becoming increasingly common in pharmaceutical analysis. In fact, this is one of the areas where robots are gradually replacing conventional techniques in control laboratories.

As an example the determination of a contraceptive in commercial tables is described, an application usually calling for a variety of preliminary operations [17]. The robot station used for this purpose includes a large number of peripherals and the operational sequence involves nearly 30 steps, the most significant of which are as follows:

(a) Placement of ten tablets in ten sample tubes.

(b) A liquid–liquid extraction with two different reagents (aqueous NaCl and chlorororm).

(c) Transfer of an aliquot of supernantant chloroform to an evaporation unit by heating in an open atmosphere with a nitrogen stream.

(d) Treatment of the dry residue with water–methanol in a sonic bath.

(e) Filtering of the supernatant through a syringe accommodating an internal fritted glass disc.

(f) Loading, capping and placement of the chromatographic vial on the chromatograph sampler.

The results obtained are comparable to and in some respects even better than those provided by manual systems.

### 9.5.4 An acid–base titration

This is an example of little practical use, but demonstrates the versatility of robotic configurations and their capability of adaptation to a variety of situations and requirements [14]. It involves the titration of a concentrated solution of hydrochloric acid with a solid basic standard, namely tris (hydroxymethyl)aminomethane (Tris).

The basic components of the station, as shown in Fig. 9.9a, are as follows: a microprocessor (Apple II computer), a single-handed robot, several measuring devices (pH-meter, pipette/burette) and a dilution unit. The operational scheme of this set-up is illustrated in Fig. 9.9b: there are four fixed positions (H-1 to H-4), one of which (H-2) accommodates a magnetic stirrer, and another six positions in which the beakers or flasks can be positioned.
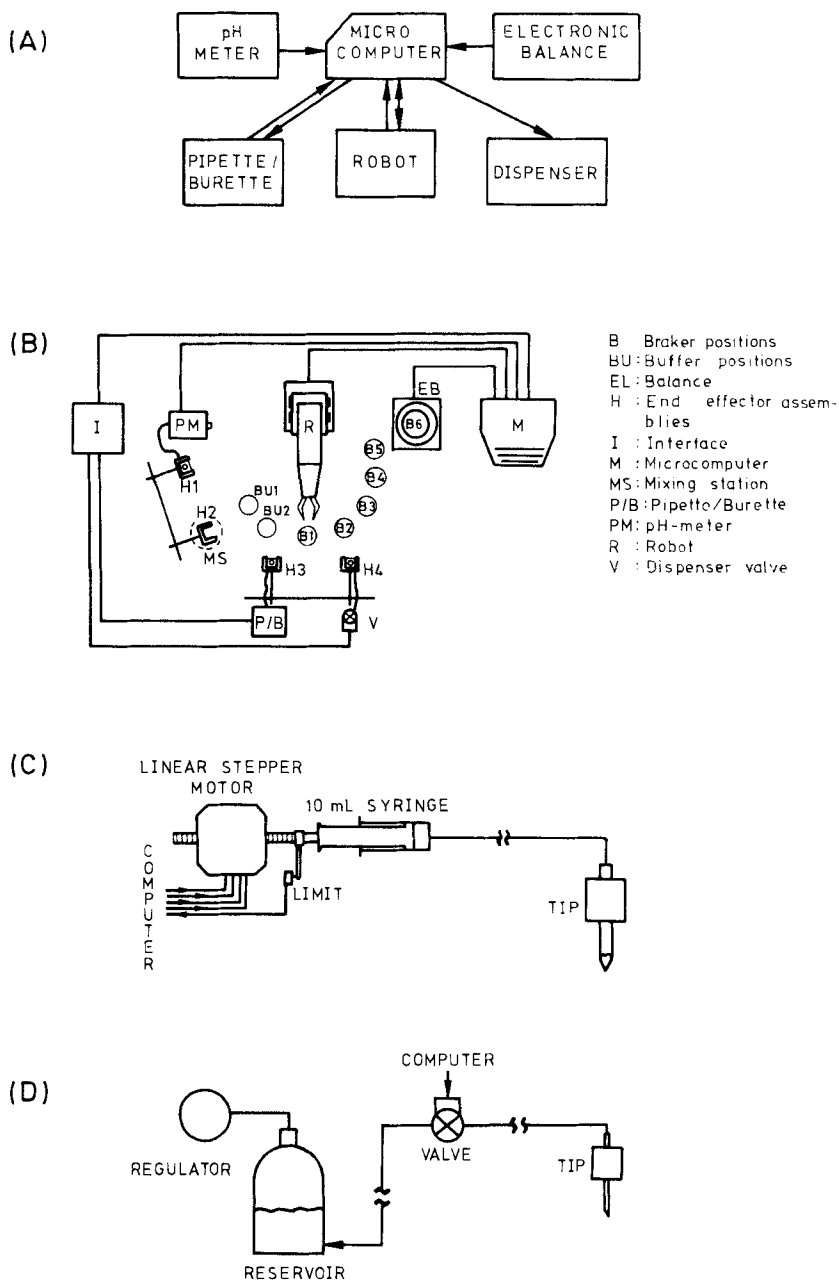
**Fig. 9.9** Robot station for titration of an acid solution with solid basic standard. (A) Block diagram of the station. (B) Top view of the situation of the different elements of the station. (C) Pipette/burette assembly. (D) Solvent dispenser system. (Reproduced from [16] with permission of the American Chemical Society).

The 18 sequential actions involved in the titration can be summed up in five:

(a) Weighing of the solid standard.

(b) Dissolution of the standard (addition of water followed by stirring).

(c) Withdrawal and dilution of an aliquot of sample (HCl).

(d) Placement of the beaker containing the standard solution in the titration position, where it is stirred and the combined pH electrode is introduced.

(e) Programmed addition of the diluted solution of the unknown in 0.2-mL increments down to pH 7 and again in 0.2-mL increments down to pH 2, at which the titration is finished.

The printer provides both the titration curve and the concentration of the HCl solution. The precision for five determinations of the same sample is not very good ($\pm$ 0.6%) compared with that achieved by the conventional volumetric method ($\pm$ 0.1%). This is typical of robotic methods.

## 9.6 SCOPE OF APPLICATION

As stated above, laboratory robotization is of especial relevance to the automation of the so-called preliminary operations, particulary when these are lengthy, cumbersome or hazardous.

Of all robot stations reported so far, the most frequently used are those handling liquid or gas chromatographs, although a number of workstations based on the use of other measuring instruments such as photometers, fluorimeters, pH-meters, and atomic and NMR spectrometers have also been described.

Among the different apparatus employed in robotic stations, vortex mixers and systems for addition/tranfer of liquids are by far the most common. Others, such as *liquid-liquid* and *solid-liquid* extractors, centrifuges, heaters and pneumatic crimpers are less frequently used, although not uncommon in robotic configurations.

Pharmaceutical analysis [18-20] has undoubtedly been the most receptive field to robotic applications, probably due to the suitability of robots for quality control operations. To a much lesser extent, robots have been used in environmental monitoring, in the treatment of biological samples (clinical chemistry) and in elemental organic and inorganic analyses. It is worth pointing out the small number of references available on the use of robotic stations for the analysis of foodstuffs or materials of industrial interest.

A compilation of the references on the application of robots in the laboratory can be found at the end of this chapter. It must be admitted that robotics is an atypical technique in this sense; in fact, although the first

robot stations were reported over 5 years ago, few papers have been devoted to this exciting subject during this period. This can be understood after looking closely at the source of the few articles published so far: most of the authors concerned work for large pharmaceutical or petrochemical firms, which are usually endowed with better means and facilities than universities or public research institutions. Not until such centres have included laboratory robotization among their priority topics and made plans for multidisciplinary research will this methodology gain due support.

## 9.7 FINAL CONSIDERATIONS

This last section poses and answers a few questions about the role that robots play and are expected to play in laboratories in the near future.

To begin with, questions such as "What is the use of robots?" and "Are there any reasons to justify the use of robots in the laboratory?" can only be answered by considering some of the advantages offered by robotized methods as compared with both manual and other automated methods, namely:

(a) Decreased costs. It has been calculated that the replacement of manual with robotic work results in a reduction in expenses by a factor of four.

(b) Increased working capability. Robots can work non-stop 24 h a day, 7 days a week.

(c) Increased reliability as a result of avoiding alterations introduced by human errors.

(d) Improved personal safety. Robots are invaluable substitutes for humans in handling hazardous samples, reagents or instruments.

(e) Flexibility. Robots allow for automation of a series of operations such as weighing, dissolution, grinding and centrifuging, none of which is affordable by other methodologies as stated above. This is of particular importance in handling solid samples.

(f) Easier adaptation of manual methods compared with other methodologies (both batch and continuous).

An apparent disadvantage of robotized methods with respect to other automatic methods is their slowness. The number of samples that can be processed per hour or day is considerably smaller, especially in contrast to non-segmented continuous methodologies such as flow injection analysis. Strictly, both extremes are not comparable insofar as robotized methods allow for automation of cumbersome, time-consuming operations unaffordable by the rest. Should the sampling rate include the time spent on preliminary operations, robotized methods would compare more favourably with other methodologies. A true disadvantage of such methods is their lower precision compared with manual procedures.

When is it really recommendable to use a robot station in the laboratory? This question is very important in considering the renewal of laboratory instrumentation and when changing the working philosophy, as it involves quite a different concept. Adressing such a compromising question requires A distinction to be made between flexible automation, characterized by its great versatility and adaptability to different situations and needs, and dedicated automation, namely the design of a specific system for a particular application.
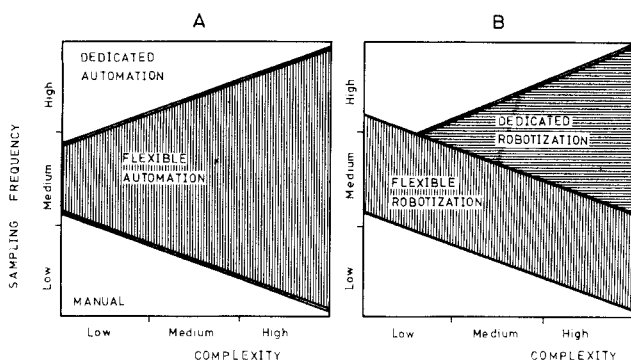


**Fig. 9.10** Suitability of different automatic methodologies according to their sampling frequency and the complexity of the analytical procedure used. (A) Without robots; (B) With robots. (Courtesy of Zymark).

Figure 9.10 shows two graphs representing the qualitative relationship between the complexity of the methodology (number of stages, precision needed, time of operation, etc) and the sampling frequency: high ($>100$ h$^{-1}$), medium ($25-100$ h$^{-1}$) and low ($<25$ h$^{-1}$). Only the graph on the left corresponds to the use of robots [22]. When the sampling frequency required is not very high and the methodology involved is uncomplicated, manual methods are preferable. Flexible automation has a very wide scope of application which justifies its use in many cases. On the other hand, dedicated automation is of use only when high sampling rates are required and not very complex methodologies are to be applied (Fig. 9.10A). The use of robots applies in rather different situations. Thus, dedicated automation and the manual methodology are equally applicable here. The zone of flexible automation is now split into two areas, one above and another below that of the manual methodology, which correspond to the use of versatile and dedicated robotic systems, respectively. The lower of these two zones covers the most compromising situations, namely high sampling frequencies and very complex analytical methodologies.

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# 10

## Automation of analytical instrumentation. I. Spectrometric techniques

### 10.1 INTRODUCTION

As stated in Chapter 1, the relationship between an analytical technique and automation can be considered from two points of view. On the one hand, analysers may incorporate a detector as a modular or non-modular element (e.g. a colorimeter in discrete analysers, a fluorimeter or voltammeter in FIA systems, a gas chromatograph in robot stations). On the other hand, automation may affect the instrumental technique itself. This is the subject matter of this chapter and the next two, devoted to optical, electroanalytical and chromatographic techniques, respectively, the instrumental alternatives most commonly used at present.

The automation of an instrumental technique can be aimed towards three different aspects, depending on whether its goal is to reduce or to completely eliminate human intervention: (a) the functioning of the different elements making up the instrument; (b) data acquisition and treatment and delivery of results; (c) both aspects simultaneously. In addition, automation can be accomplished to different degrees depending on the number of modules or elements or the operations involved. Built-in and on-line coupled microprocessors play a major role in automation. The vast development and technical and economical affordability of hardware and software in the present decade have revolutionized instrumental analytical chemistry, considerably improving the performance of classical instruments and even facilitating the development of new instrumental modes.

This chapter deals with a selection of the most significant achievements and trends in the automation of spectroscopic techniques —a comprehensive description would require a separate book. In addition to the automation of the functioning of the spectrometer and the acquisition and treatment of data, it discusses the different alternatives for the identification and/or elucidation of organic structures through computer-supported data bases.

### 10.2 AUTOMATIC CONTROL OF THE FUNCTIONING OF AN OPTICAL INSTRUMENT

The optical instruments most commonly used in laboratories (spectrophoto-

meters, spectrofluorimeters, different types of atomic spectrometers) are increasingly being subjected to automation. The advances in the automatic control of the different modules involved are continuously being incorporated by manufacturers in their latest instruments, of which there is a wide range currently available.

Below are described separately the automation of each of the modules making up an optical analytical instrument and that of the instrument as a whole.

### 10.2.1 Control of the light source

The automation of the light source involves two aspects: the control of the lamp changeover and that of the stability of the source proper. Lamp changeover (from UV to visible or vice versa) in a spectrophotometer is very easy and is effected through a change in the monochromator position. There is a critical wavelength which, according to the direction of motion, starts one or the other lamp. A single-bit signal is enough for the computer to trigger either lamp.

Figure 10.1a depicts a circuit for control of the stability of the light source by means of an analogue-to-digital converter that compares voltages with a precision to within 50 μV. Such precise integrated circuits are not affordable, so the common solution is manual adjustment or, even better, the use of an automatically self-adjusting circuit such as that shown in Fig. 10.1b. This microprocessor-controlled circuit performs alternate auto-zero and measurement cycles, thereby effectively eliminating the offset adjustment. Its performance is improved by a reduction in the effect of temperature on the offset voltage drift [1].

Pardue and co-workers demonstrated that the "optical feedback" concept could be used in developing very stable single-beam photometers [2,3]. In these systems, a portion of the light beam is diverted by a beam splitter to a control detector, which generates a signal that is used in a programmable power supply to control the source intensity. Any drift in intensity is detected and used to control the power to the source to compensate for the drift. In subsequent work [4], Rossi and Pardue [4] evaluated the usefulness of the optical feedback principle with a multi-wavelength imaging detector. The results obtained in the visible region with a tungsten source were reported to be very encouraging. Reproducibility data for the system with feedback were more than one order of magnitude better than for the system without feedback for 13.5-s, 5-min and 1-h measurement intervals. The results with feedback compared favourably with those obtained with a commercially available dual-beam system. Such results showed that the combination of optical feedback with the diode array detector can provide the combined advantages of rapid-
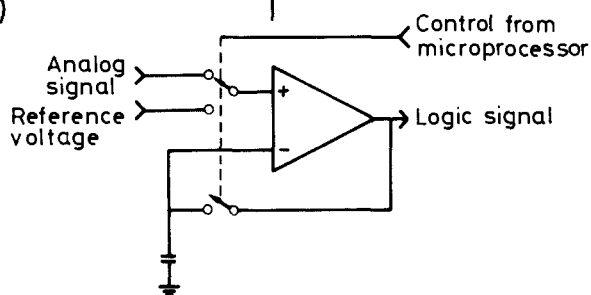
scan spectrophotometry with long-term stability. This capability should further enhance the utility of these detectors.

a)
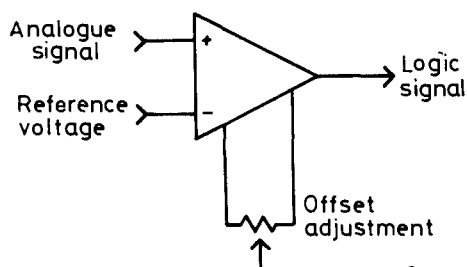


b)



**Fig. 10.1** Circuit of analogue-to-digital converter for control of light source stability. (a) Manual offset adjustment. (b) Automatic self-adjusting circuit. (Reproduced from [1] with permission of International Scientific Communications).
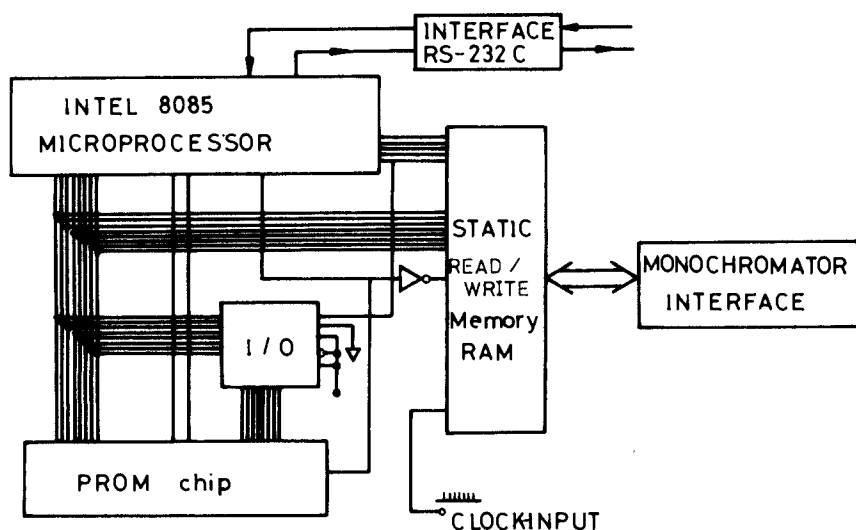
### 10.2.2 Control of the monochromator

The use of high-resolution grating monochromators for the isolation of the selected wavelengths is of crucial relevance to spectrochemical measurements. The remarkable advances in the functioning of monochromator control systems have considerably improved the precision with which these units can select a given wavelength.

Dolle-Molle and Defresse [5] proposed the use of a microcomputer furnished with an Intel 8085 microprocessor to control a spectrophotometer monochromator. The microprocessor can be programmed to control the monochromator movements and perform scans in linear energy units while storing in memory the correction (energy increment) to be introduced at each wavelength. Figure 10.2 shows the scheme of the microcomputer used. The program controlling the CPU is stored in the PROM (programmable read-only memory). The I/O unit controls the

access to the sentences of the program, which are transferred in due course to the CPU through bytes (8-bit parallel reading). These CPU-PROM (AD0-AD7) lines are bidirectional, while the other two CPU-PROM lines and the inversion CPU-RAM line are only unidirectional (output) and function to drive the highest-order byte to the memory addresses. The RAM is used to store the variables associated with the program controlling the monochromator. The programmable timer accommodated in this chip is used to generate the wave pulses required to control the stepper motor fitted to the monochromator. The correction factors used by the monochromator program [6] are obtained by determining the emission maximum from the average of five measurements of each atomic calibration line. The spectral lines are measured for various types of lamps at different times and roughly the same temperature. The data are treated by means



**Fig. 10.2** Scheme of the microcomputer used to control a spectrophotometer monochromator. (Reproduced from [5] with permission of Taylor & Francis).

of a purposely written program for minimization of the weighted sum of the squares of the residues, from which the above-mentioned correction factors are calculated.

### 10.2.3 Control of the sample compartment

Spectrometers with several sample compartments that can be sequentially aligned with the optical path allow for the control and programming of this unit via a computer. The compartment movements are effected by a small stepper motor whose different positions are governed by the computer via a suitable interface. Papoff and Ricci [7] proposed the use of the standard Multibus IEEE 796 for this purpose. The scheme of the configuration and its actuation on the sample compartment tray is illustrated in Fig. 10.3. The microcomputer uses an internal Multibus interface to communicate with the standard IEEE 796 interface, which contains two independently addressable spaces: one for the memory and another for input/output instruments which is linked to three programmable timers (the motor is a four-step one), a programmable peripheral interface and a programmable control switch. Each timer consists of three independent 16-bit counters working in six different modes. The mode and count of each counter can be programmed by means of software. Two counters are used for each step of the motor (step time and number). The peripheral interface determines the direction and selection of each motor step. The control switch warns the computer about the end of each operation and allows the synchronization between the
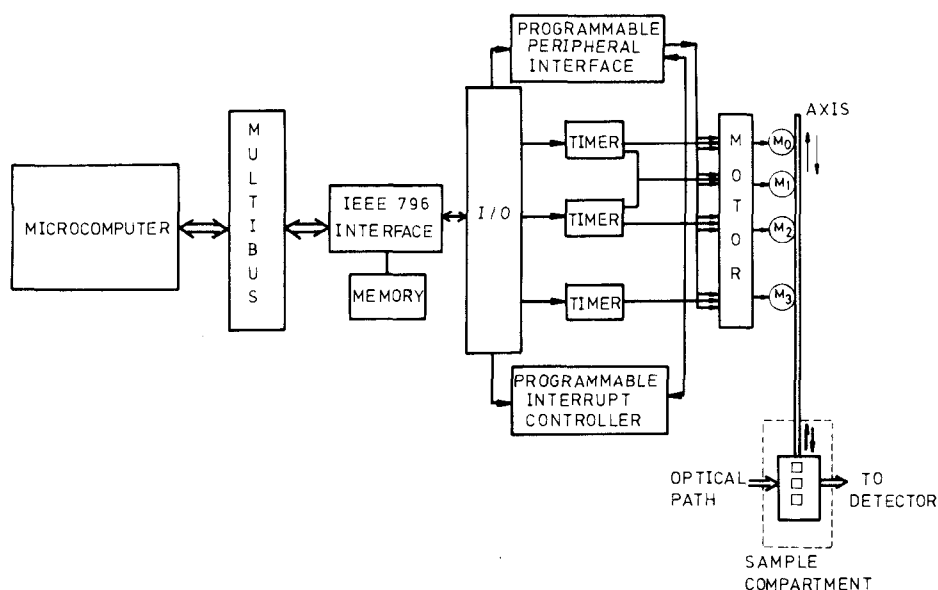


**Fig. 10.3** Scheme of automatic computer-controlled spectrometer sample compartment. (Reproduced from [7] with permission of Pergamon Press Ltd.).

software and the mechanical operations involved. Each of the four possible positions of the sample compartment is selected by a motor step through a shift along the compartment axis. The stepper motor can be successfully employed in the automatic control of chemical instrumentation whenever required, and was used in a computer-controlled spectrometer combined with an inductively-coupled plasma source, designed for automatic sequential determinations [8,9]. In the spectrometer, the controller handles: (a) two stepper motors for the wavelength indexing in an échelle monochromator (where one motor controls the grating and the other the prism); (b) a stepper motor-controlled rotary mirror for the selection of the proper observation zone within the plasma plume; and (c) a stepper-motor peristaltic pump for control of sample flowrates in the plasma nebulizer [10].

Positional stability has been shown to be an important parameter in determining the analytical performance of the high-voltage spark discharge. Realtime monitoring of the positional stability of the spark is therefore a useful tool in the quality control of spectrochemical analyses. The goal of the work reported by Stewart and Scheeline [11] was to build a device that could monitor the position of every spark in a train. As typical determination procedures may involve thousands of sparks, it quickly became evident that some data reduction would be necessary before the storage of individual spark positional data. Without some compression, the data from the 256-element diode array employed would quickly fill all 64 kb of memory available on an LSI-11 computer. The hardware reported required digitization of only two numbers per spark and was successfully used to observe a train of 2000 sparks. The duty cycle was sufficiently slow (no more than 720 digitizations per second), so the computer could have performed other tasks simultaneously. The device demonstrated its capability to compute the centroid position of a non-point light source or shadow. One of its applications involved the monitoring of the position stability of a train of spark discharges. The device could be employed for on-line monitoring of the positional stability of a spark or arc during routine analyses. By condensing raw video data before digitizing for computer storage, positional data for entire trains of sparks could be realistically stored.

### 10.2.4 Overall control of the instrument

Virtually every optical instrument commercially available at present accommodates a microprocessor which, through a small keyboard, allows the user to select the different instrumental variables and programming the instrument's functioning during the analyses. More recent and sophisticated instruments have no keyboard and are controlled via a screen on which the working modes and parameters are selected by means of a 'mouse'.

Typical examples of microprocessor-controlled instruments are the UV-visible spectrophotometers of the Lambda Series, marketed by Perkin Elmer. Figure 10.4 shows the scheme of the simplest instrument in the series, the Lambda 1. Its microprocessor consists of an analogue and a digital section which constitute the heart of the instrument, or CPU, which contains the control programs (microprocessor and PROMs), and different integrated circuits for transmittance-absorbance and absorbance-concentration conversions and other functions. There is a series of peripherals for information output and display, and a keyboard that allows the user to key in instructions for the digital control unit, which in turn acts on a filter to select the lamp (tungsten or deuterium) and control the monochromator (wavelength), both of which are accomplished through a stepper motor. Other models, such as the Lambda 5, are dual-beam and feature a microcomputer with a larger menu.
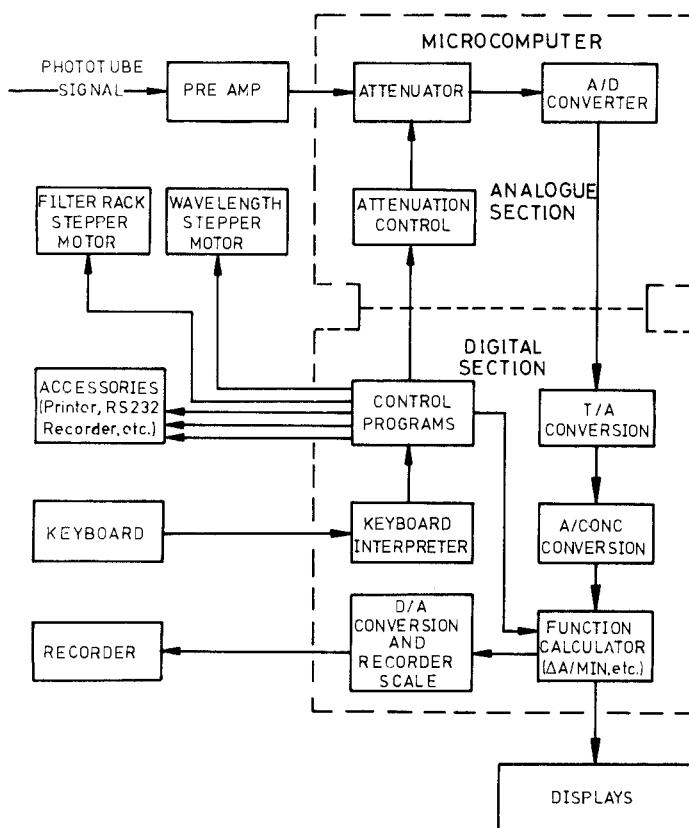


**Fig. 10.4** Lambda 1 microprocessor-controlled UV-visible spectrophotometer Lambda 1. (Courtesy of Perkin Elmer).

The Spectronic 501/601 is a basic and flexible microprocessor-controlled UV-visible spectrophotometer [1]. The instrument control is accomplished through an extremely user-friendly keyboard with readily comprehensible labels. An alphanumeric display presents commands and messages to tell the user what the instrument is doing, what action is required and what parameters are allowed by the system. Table 10.1 illustrates the various functions accessed by the keyboard. Data appear on the display and can be sent to a computer or a printer. A strip-chart recorder can provide hard copy of data collected over time. The modular design of the instrument, shown diagramatically in Fig. 10.5, contributes to its versatility. A variety of accessories for sample and data handling can be interfaced with the spectrophotometer, enabling users to customize it to suit specific requirements. Expansion of capabilities as finances allow or as needs demand is thereby also possible. Electronic control of sample handling takes place through the accessory control module (ACM), which also allows one-way data communication to an external printer or two-way communication to an external computer via an RS232C interface. Additionally, the ACM accepts APPLIPAK program cartridges for control of automated accessories and for data manipulation. The CAM directly or indirectly controls a variety of accessories and processes. The APLIPAK cartridge design concept offers a number of major features. Because the cartridges are separate units, they can be removed from the ACM and stored in a desk or be sent, already programmed to another laboratory. With the appropriate adapters, long path rectangular or cylindrical cuvettes and test tubes can be placed in the single- or
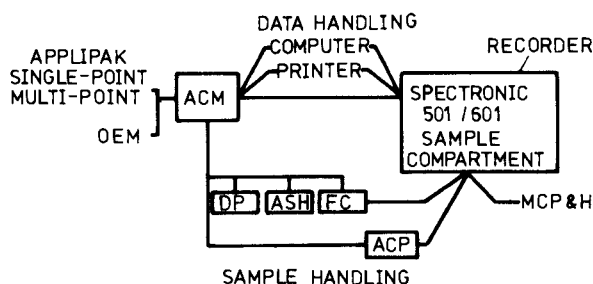


**Fig. 10.5** Modular configuration of the Spectronic 501/601 spectrophotometer. ACM: accessory control module; OEM: original equipment manufacturer; DP: diluter-pippeter; ASH: automatic sample handler; FC: flow-through cells; ACP: automatic cell positioner; MCP and H: manual cell positioners and holders. (Reproduced from [1] with permission of International Scientific Communications, Inc.).

multiple-position cell holders. The ability to accommodate long path cells is important in applications where high sensitivity is required, such as in environmental or water research.

**TABLE 10.1**

Keyboard-controlled functions of the Spectronic 501/601

| Function | Description |
|---|---|
| Data mode | %T, absorbance, concentration |
| Wavelength | User specified: 325-999 nm (VIS); 195-999 (UV) |
| High and low limits | Flags tests values outside user-specified range |
| Auto zero | Sets zero absorbance or 100%T |
| Signal average | Three levels: 0, 1, 2 |
| Lamp: | |
|   Lamp save option | On or off |
|   Tungsten or deuterium Lamp interchange | On or off |
|   wavelength | 325-380 nm |
| Multiplication factor | Converts absorbance to concentration |
| Control of an accessory | Set RS232C interface parameters (baud rate, parity, etc.) |
| Test parameter storage | Programmed parameters become "default" values |
| Self-test | Verifies proper function of major electronic and mechanical systems |
| Error messages | Instructs operator and identifies part malfunctions |
| Diagnostics | 16 user- and 25 service-specified function checks |
| APPLIPAK cartridge | Loading, editing, listing, storing and cataloguing of programmed tests |

The case with spectrofluorimeters is similar: the microprocessor controls the emission monochromator and, optionally, the excitation monochromator (or filters), but it also controls the power supply to the xenon lamp used as the light source through a reference signal from the photodiode. In addition, the microprocessor can compute the information from the detector. A representative example of a computer-controlled spectrofluorimeter is the Jasco FP-770 (Fig.

10.6). The control of this instrument is in accordance with commands originating in the data station unit (DSU). Among the features of the optical unit are a variable spectral bandwidth in ten steps from 1 to 1000 nm/min, a software-controlled shutter that closes the aperture of the excitation monochromator and an auto-zero function. A fast response dynode-feedback technique is employed to compensate for characteristics of the light source and the excitation monochromator, and also light source fluctuations. The dynode-feedback technique is incorporated within the reference optics and is adapted to control the photomultiplier high voltage electronically. By changing the gain of the feedback loop in four steps, the technique can effectively cover a wide dynamic range of incident radiation. The FP-770 takes advantage of the microprocessor in both instrument control and data manipulation. The DSU consists of three modules: a 12-in, high-resolution cathode ray tube (CRT) display, a thermal graphic printer/plotter and a processor module which houses microprocessors, memory, digital bus and electronics; 514 kb of read only memory (ROM), random access memory (RAM) and battery back-up memory can be housed within the processor module. To maximize the data throughput, the FP-770 employs two salve processors which aid the master processor by sharing relative priority jobs. Regarding the software structure, the instrument uses the
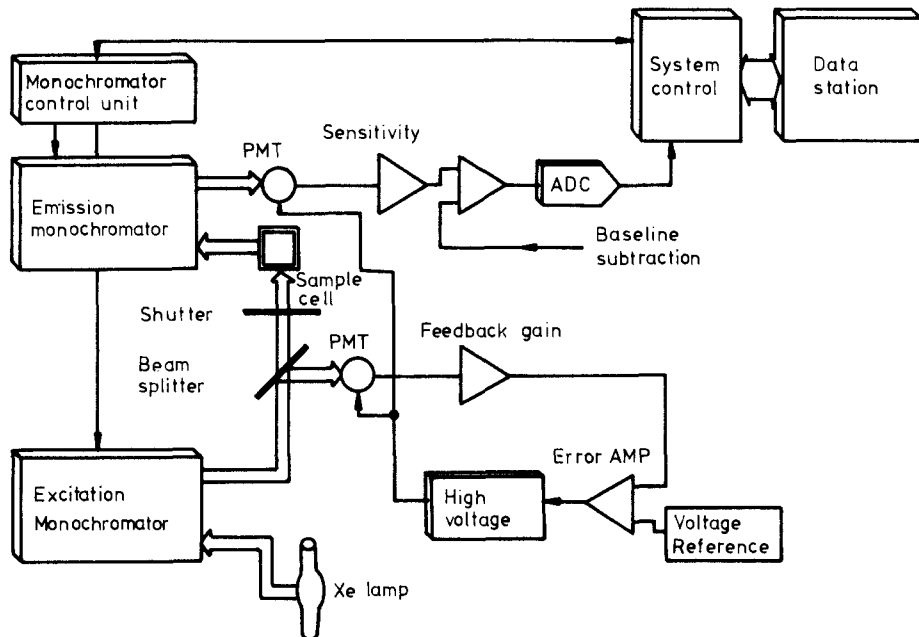


**Fig. 10.6** Block diagram of the Jasco FP-770. (Reproduced from [10] with permission of Japan Spectroscopic Co., Ltd.).

multi-bank operating system (MOS) to allow real-time capabilities for instrument control and data manipulation. The DSU can physically address up to 1 Mb of memory, which is divided into 16 banks of 64 kb memory. Each memory bank is mapped every 4 kb and is assigned different specific tasks. Task 1, or the main task, is assigned to Bank 1. The other tasks, which control the real-time data, graphic and plotter displays, are assigned to banks 2 to 4. Menu-driven operation enhances ease of operation. Each set of programs for a particular application is labelled with a specific 'method' number so that the operator can readily select the desired program. Each method has two pages: a parameter page on which the setting of parameters, decision making and data acquisition are performed, and a measurement page, where the actual measurement of the sample is carried out once the instrument conditions have been determined on the parameter page. User-friendly software allows the operator to determine the instrument conditions by means of interactive communication with the instrument through the screen [10].

Atomic methods also benefit from the incorporation of microcomputers into the instruments. Figure 10.7 shows the scheme of an atomic absorption spectrometer with a built-in microprocessor which controls the signal from the detector, previously amplified and converted to digital form. A series of ROMs store the programs for zero-setting, calibration, statistical treatment and calculation of integration areas and times. The microprocessor modulates the
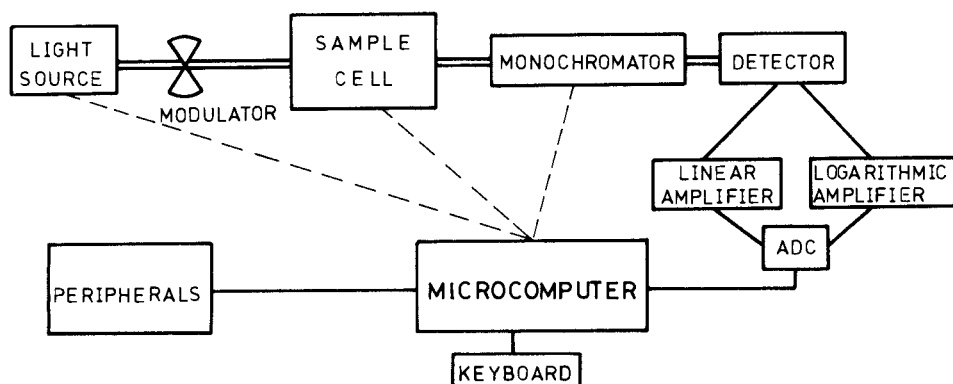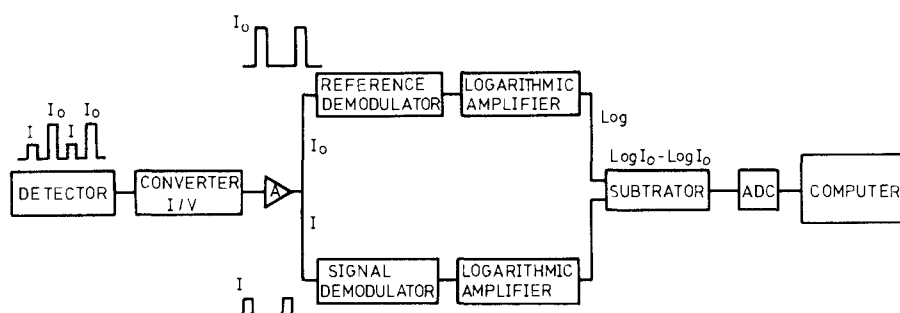


**Fig. 10.7** Scheme of an atomic absorption spectrometer incorporating a microcomputer.

lamps and the detector, controls the shutter rotation speed in dual-beam spectrophotometers and performs various conversions of the analytical signal. In

systems with background correction, it discriminates between and controls the radiation from the primary (I) and the continuous (Io) source (Fig. 10.8) [12]. The signal from the detector is converted to a voltage and amplified. The signals I and Io are then split and separated as a function of time and the modulator rotation speed, controlled by the computer. Once the signal has been demodulated and amplified in a logarithmic form, a subtractor converts it to absorbance units which, after digitization by an ADC, are processed.



**Fig. 10.8** Processing of the electronic signal from the detector in an atomic absorption spectrometer with computer-controlled background correction. (Reproduced from [12] with permission of Academic Press).

In electrothermal atomization methods, the microcomputer also controls the furnace temperature, a key factor for this technique. Guevremont and Whitman used a microcomputer based on a Z80 microprocessor for the automatic control and data acquisition from a graphite furnace [13]; they could heat the furnace from 0 to 2500°C in about 3 s, with an approximate gain in precision of 50% under the control of the microprocessor. The system is shown schematically in Fig. 10.9. The automatic functions afforded are data acquisition, measurement of the furnace temperature and calibration, temperature programming, control of the gas flow, control of the sampler and delivery of results.

The computerized system consists of a Cromenco computer with a Z80 microprocessor, 64 kb of memory and access to various peripherals, and different interfaces such as a serial-parallel Cromenco TU-ART interface, 8- and 12-bit A/D and D/A converters and a parallel-reading I/O Cromenco 4 PIO unit.

The furnace temperature is measured by means of an internal current-voltage of the furnace and an external IR detector working above and below a user-

selected threshold. They are calibrated by comparing the detector signals with previously tabulated data stored in the computer's memory. The temperature program is constructed from a d.c. signal applied to resistor E123 of the original furnace control circuit. This signal can range from +15 V (furnace off) to +5 V (maximum power). A 12-bit DAC is used to minimize the difference between the programmed and actual furnace temperatures (resolution). The computer controls the furnace via this DAC. In addition to the furnace, the microprocessor controls the gas (air or hydrogen) flow that can be introduced into the furnace during a given stage of the temperature program. Such control is exerted from the keyboard through relays which act on the solenoid valves of the furnace. The microprocessor also controls the autosampler (Perkin Elmer AS-1), sending a signal each time a new sample is to be injected into the furnace. After injection, the computer detects the autosampler signal and starts the furnace temperature program.
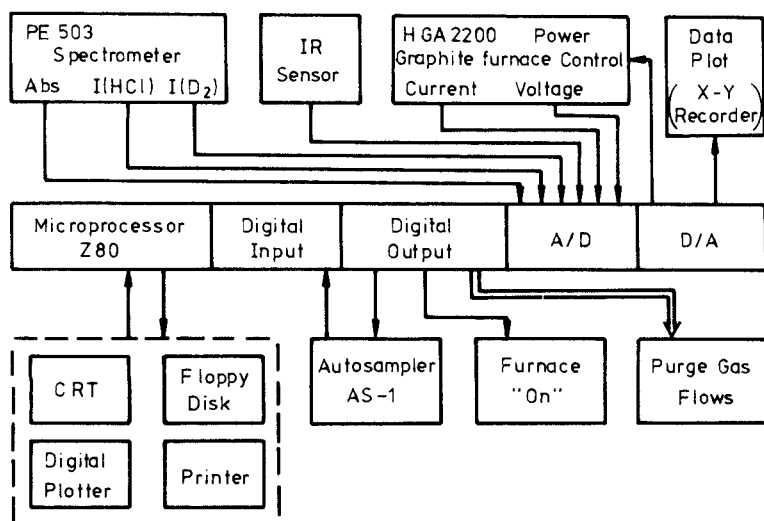


**Fig. 10.9** Automatic control of a graphite-furnace atomic absorption spectrometer by means of a microcomputer. (Reproduced from [13] with permission of Elsevier).

The design of the Perkin-Elmer Plasma II ICP Emission Spectrometer is aimed at the complete automation of the instrument's operation, which simpli-

fies its operation to such an extent that all of the analyst's activities are combined in a coherent software body, similarly to the so-called 'integrated software', so popular in the field of personal computers. All the activities in which the analyst may be involved during the instrument's operation can be processed simultaneously, which means that the operator can revise previous data, generate reports or define new analytical conditions while an automatic analysis is being performed [14]. The primary functional subsystems of the instrument are the controlling computer, two monochromators, the ICP source and the autosampler. These systems are linked together by means of an IEEE-488 communications link. All the major subsystems and the controller are daisy-chained together in a single network. The standard spectrometer configuration incorporates two independent 1-m Ebert monochromators. These comprise a unique mechanical design unit which employs one of two blazed holographic gratings. A typical configuration incorporates a grating that provides a measured bandpass of less than 0.009 nm in first order in one monochromator with a grating that gives wavelength coverage of up to 800 nm in the other monochromator. Both units may be evacuated to less than 30 mTorr for optimal performance at wavelengths below 190 nm. The monochromators, grating drives and detectors are thermostated to provide a constant thermal environment for the optomechanical system. The Myers-Tracy Signal Compensation system, an advanced internal standardization technique utilizing a dual-channel filter photometer for each monochromator, allows 5- to 10-fold improvements in precision for samples containing high concentrations of dissolved solids. Each monochromator subsystem incorporates its own microprocessor and internal memory. All optomechanical and detector components are controlled by the system controller through this microprocessor. These components include the grating drive, ICP source observation height adjustment, a six-position filter wheel, an internal mercury source, a photomultiplier and sensors for monochromator temperature and vacuum. The integrated control of two monochromators operating simultaneously, yet independently, allows analytical speeds of up to 50 elements per minute to be achieved while covering a 600-nm wavelength range.

The ICP source subsystem incorporates its own internal microcomputer and memory in order to execute the controller's commands. The RF power supply is under computer control. Likewise, all of the argon flows, the ignitor, peristaltic pump and safety interlocks are directly controlled by the system computer. The RF power output is stable to less than 0.01% over 1 h of operation. The nebulizer argon flow is controlled through a thermostated mass flow controller to ensure long-term stability. The uptake rate of the two-channel peristaltic pump incorporated within the source subsystem is variable by the system controller.

The autosampler functions as a separate IEEE-488 device, distinct from the source system. It also incorporates its own internal microcomputer and associated memory. It allows random access of any sample in the tray at any time during the analysis. Using the autosampler for sample introduction allows the analyst to perform other analysis-related tasks while an analysis is taking place. Including the system controller, both monochromators, the RF system and the autosampler, the instrument assembly contains five separate microprocessors.

The instrument controller managing all this hardware is the powerful Motorola 68000 microprocessor. This is a 16-bit device that uses 32-bit internal calculations. The system includes a 15-Mb hard disc with two 320 floppy disc drives as standard components. The controller, configured for the ICP spectrometer, contains a total of 1.6 Mb of RAM.

One of the benefits of permitting every source and optomechanical parameter to be under the control of the system software is the automation of the optimization of the instrument's operating parameters. The analyst may choose to have the instrument automatically select the optimal operating conditions for parameters such as plasma RF power, nebulizer argon flow, source observation height and analyte wavelength. Since the term 'optimal' many take on different meanings for different applications, the optimization may be carried out with respect to a number of performance criteria: signal-to-background ratio, signal-to-noise ratio, analyte peak precision and degree of spectral overlap interference. This optimization process may be performed by either of two methods. With one method, the analyst selects the specific parameter values to be evaluated during the optimization, while the other allows the instrument to select the parameter values investigated based on previous results of that parameter.

## 10.3 AUTOMATION IN THE ACQUISITION AND TREATMENT OF SPECTROSCOPIC DATA

The previous section described the reduction of human intervention in the operation of an optical instrument in order to facilitate or make possible its functioning. Another aspect of great interest in this context is the automation of the acquisition and treatment of data from an instrument based on measurements of an optical nature, understandably the earliest to be developed on account of its great simplicity.

The degree of automation of this last stage of the spectroscopic analytical process depends on a variety of factors that can be summarized as follows:

(a) The characteristics of the spectroscopic technique involved. Some of them (e.g. modes based on image detectors [15,16] or Fourier transform spectroscopy [17]) generate a large number of data simultaneously at a high rate.

(b) The analytical method applied. Thus, procedures based on reaction rate measurements [18], commonly used in the enzymatic and clinical fields, are generally implemented on configurations with automated collection and processing of the absorbance-time data pairs to deliver the results [analyte concentration(s)] as required.

(c) The level of information required, which will depend on the features of the spectroscopic technique or method applied —the higher its intrinsic selectivity and/or sensitivity, the fewer data will be needed, the simpler their handling will be and the smaller will be the need for automation— and on the complexity and quality of the results needed (single- or multiparameter determinations, variations as a function of time, etc.).

Automation in data acquisition and treatment can be aimed at a variety of objectives inherent in the above-mentioned factors. It should be pointed out that physical and physico-chemical kinetic factors play a decisive role in the reduction of human intervention. The acquisition of data at a high rate imposed by the technique itself (e.g. picosecond spectroscopy [19]) or by the system investigated (e.g. meaurements of rates of reactions with half-lives of the order of a few milliseconds by the stopped-flow methodology [20,21]) demand the use of a computerized system without which application of the particular spectroscopic technique or method would not be feasible. On the other hand, the so-called 'microprocessor-controlled spectroscopy', widely commercialized at present, broadens the scope and facilitates the operator's work by eliminating various sources of error.

From the point of view of the automation of data acquisition and treatment, spectrometric techniques must be divided into two large groups according to the manner in which the spectral information is generated, which in turn depends on the optical design of the instrument used:

(a) *Conventional spectroscopic methods*, in which the entire spectroscopic information is obtained by scanning across the spectral region of interest by using a continuous source and a dispersive element (a grating or a prism) which is mechanically rotated to vary the wavelength of light passed through a fixed exit slit. The resulting monochromatic light passes through the sample cell and is detected by a photomultiplier tube. Because sampling is limited to a very narrow wavelength range, data points are acquired at different times. The use of a computer for data acquisition and treatment is not strictly indispensable except in the exceptional cases commented on above, although its incorporation offers substantial advantages with respect to manual alternatives.

(b) *Multiplex spectroscopic methods*, in which the complete spectroscopic information is obtained by simultaneously monitoring the spectral region of interest throughout. They are also known as 'multi-wavelength detection meth-

ods'. In addition to the rapid refinement of semiconductor-based technology, the performance of these methods is based on the affordability of powerful microprocessor systems, without which they would be virtually unfeasible insofar as they require an automatic system for discrimination, storage and delivery of data in a fast manner suited to these simultaneous methodologies. This classification is used here as the basis for the description of some of the more general possibilities of automation in the acquisition and processing of spectroscopic data.

### 10.3.1 Conventional spectrometric methods

UV-visible spectrophotometers marketed by most manufacturers in the last few years have incorporated a microprocessor. The traditional appearance of these instruments has changed, the microprocessor keyboard replacing the classical buttons. A UV-visible spectrophotometer with a built-in microprocessor allows the automatic selection of a number of parameters: e.g. transmittance, absorbance, concentration, and first, second and third derivatives. The microprocessor provides for automated baseline storage and compensation and even for diagnostics. Information concerning the lamps (tungsten and deuterium) can be digitally displayed and also permanently recorded on a built-in printer. By means of a straightforward program it is possible to directly obtain the results of photometric methods based on reaction rate measurements [22]. Because the exact conditions under which a sample is run can be duplicated a day, a month or even a year later, the time reproducibility should show much improvement over manually operated instruments. In addition, the results should also show greater operator independence. As a rule, the manufacturer offers bundled software suited to the instrument's potential and requirements. Nevertheless, a variety of programs [23,24] suitable for the on-line coupling between a computer and an ordinary spectrophotometer have been reported [23,24]. Such is the case with the configuration shown in Fig. 10.10 [24]: a Varian DMS 90 UV-visible spectrophotometer with an HP 9854B desk-top computer coupled with a multi-programmer (HP 6940B), equipped with two 16-bit parallel I/O interfaces, an IEEE48 serial I/O interface and a 12-bit A/D converter. The digitizing rate is controlled by a real-time clock. The parallel input of the spectrophotometer is coupled with a parallel I/O interface of the computer (connector EXT KDB, D0-D5 and GND in Fig. 10.10). Signal acquisition was first tried over the IEEE48 I/O bus of the spectrophotometer, but failed because the protocols of the control lines were not compatible. Therefore, the A/D converter in the multiprogrammer was used to read the recorder signal of the spectrophotometer. An advantage is that a high digitizing rate is achievable, allowing signal averaging. The program package consists of ten subroutines and functions

written in BASIC. The routines are written in a top/down structure, meaning that at the highest level the more complex routines are found, which calls lower level routines down to the most primitive ones. The user has only to deal with the highest levels and to initialize some strings and variables. The program structure is as follows: level 3 (main section); level 2 (subroutines Init and Step); level 1 (subroutines Frame, Save, Load and Ask-input, and function FNWave).
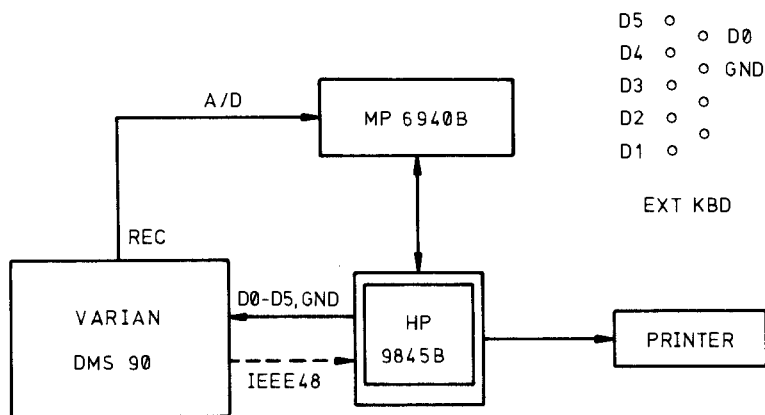
**Fig. 10.10**  Scheme of the hardware of spectrophotometer-computer coupling. (Reproduced from [24] with permission of Elsevier).

The need for the on-line incorporation of a computer for the acquisition and treatment of the kinetic data generated by a stopped-flow instrument couple to an optical instrument (generally a UV-visible spectrophotometer) was established no less than 20 years ago [25]. The variety of hardware and software used for that purpose is an illustrative example of the evolution of the different types of computers, continuously superseding previous models in performance [20,21,26-31].

In contrast to the well-known difficulties of traditionally applied quantitative IR spectroscopy of mixtures in solid (powdered) samples, the near-infrared reflectance analysis (NIRA) technique [32] has gained importance over the last decade and can now be implemented on a variety of commercially available instruments in a number of applications to industrial, agricultural and pharmaceutical analyses. Both the NIRA instruments equipped with grating monochromators and those fitted with filter systems feature built-in microprocessors with software suited to the intrinsic characteristics of this spectroscopic alternative. Filter instruments generate raw optical data for only a few wave-

lengths in two types of optical mountings: the continuous moving filter mount and the turret wavelength section of discrete narrow band interference filters. There are three levels of filter instruments (minimal, basic and enhanced) from which to choose for analysis by multi-channel discrete wavelength measurements and computer data treatment. Enhanced systems have auxiliary computer hardware to allow rapid in-house custom calibration and method development. Other channels or wavelengths may be added to or substituted for those commonly used on the basic system. The system may allow direct raw data transfer to a computer equipped with statistical software suitable for identifying the channels best correlated to the amount of analyte of interest by the t-test or other statistical functions. Statistical terms and calibration coefficients typically result from multiple linear regression of data from a suitable training set. Such a set is selected to include a suitable range of analyte concentrations in a matrix containing other constituents in a typical variation cross section for that product. Good reference classical laboratory analytical data are a prerequisite for success.

Molecular fluorescence spectroscopy has clearly benefited from automation through the incorporation of microprocessors built into or coupled on-line to the spectrofluorimeter. Such is the case with the Perkin Elmer MPF-66, controlled by a PE 7000 Series professional computer, which has a 16-bit microprocessor with 640 kb of RAM, a 10-, 15- or 28-Mb Winchester disc and two floppy disc drives each with 320 kb of data storage. Williams [33] wrote the integrated software package for this configuration, which includes a variety of useful routines, many of which are new in the field of fluorescence spectroscopy. The software commands available allow the operator to perform the following functions: (a) control the spectrometers; (b) acquire spectral, kinetic or chromatographic data; (c) store and retrieve on the floppy and Winchester discs; (d) manipulate data; (e) display data; and (f) automate measurements by using OBEY functions. In an interesting paper, Warner *et al* [34] systemized and stressed the usefulness of multidimensional luminescence measurements (spectral, polarization, lifetime and miscellaneous approaches) in improving and expanding the potential of conventional fluorimetry. These approaches can be implemented on a conventional spectrofluorimeter or by use of image detectors. The most important aspect in both instances is data acquisition, display and processing in a multidimensional format (e.g. fluorescence intensity-excitation wavelength-emission wavelength and fluorescence intensity-emission wavelength-time graphs, contour plots), for which the computer is an irreplaceable aid.

The incorporation of microprocessors into atomic emission or absorption spectrometry is now a fact in most current commercially available instruments.

Self-contained microcomputers are frequently interfaced by means of a digital communications link to an analytical instrument with a built-in micropro- cessor. Often the digital values are obtained at the end of the analogue processing chain and are thus dependent on the operating mode of the instru- ment. Lum *et al.* [35] reported a universal interface for the rapid acquisition of raw analogue data from virtually any atomic absorption spectrometer using a basic microcomputer (Apple II). As shown in Fig. 10.11, it consists of an elec- tronic circuit for analogue signal conditioning and integration, an analogue- to-digital converter (12-bit resolution, 0.78 ms conversion time) and a program written in 6502 machine code. For operation with a Perkin Elmer 5000 (or 4000) atomic absorption spectrometer, three signals from the instrument are used: (a) the 'read' signal available at an external connector, (b) a 50-Hz square wave (jumper SP4, analogue receiver board), optically coupled to the chopper rotation, and (c) the output of the photomultiplier preamplifier (TP3, analogue receiver board). Signals (a) and (b) are buffered and applied to single-bit (PB) inputs of the Apple I/O connector. The state of these inputs is monitored in and controls the execution of the program. During the measurement period, which begins when the state of PB2 changes to low, 'hold'
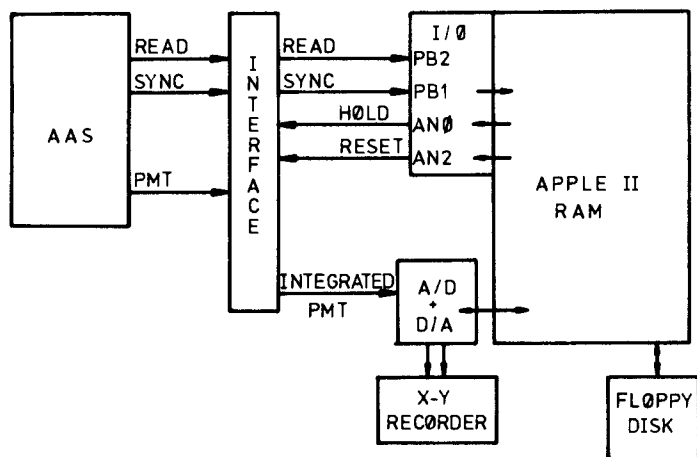


**Fig. 10.11** Block diagram of interface for atomic absorption spectro- meter with a basic microcomputer. (Reproduced from [35] with permis- sion of Elsevier).

and 'reset' pulses synchronized with the 50-Hz square wave are generated at annunciator (AN) outputs by program instructions. These pulses are timed by means of programmed delays to coincide with the integration intervals. All values obtained for each 20-ms cycle during an observation period of up to 20 s are stored and may be transferred to disc for subsequent recovery and/or manipulated after the completion of the measured cycle. This interface can be adapted to a variety of specific applications by means of minor modifications to the software. Intensity variations resulting from both atomic and non-specific absorption are monitored with a time resolution limited only by the inherent characteristics of the instrument. These data are then manipulated to produce individual plots of line absorbance and non-specific absorbance, in addition to 'corrected' atomic absorbance *vs.* time. The use of the interface in no way affects the operation of the spectrometer. The data acquisition system was developed for a study of matrix modification methods for the reduction of spectral interferences in the determination of selenium and arsenic by graphite-furnace atomic absorption spectrometry.

Mass spectrometry has also benefited from the incorporation of computers since the second half of the past decade. Current commercially available mass spectrometers are highly automated, which facilitates their operation enormously and clearly widens their potential. As a rule, manufacturers supply their own bundled software —frequently copy-protected— suited to their computer-controlled instruments and difficult to access by the user. Nevertheless, several customized on-line couplings between different types of mass spectrometers and computers have been described. The example illustrated in Fig. 10.12 is based on long experience at the Max Plank Institut für Kohlenforschung [36]. In order to satisfy the requirement of servicing several spectrometers simultaneously, the application of separate computers was the most appropriate solution: a satellite processor dedicated to a single spectrometer could handle all high-priority tasks related to the acquisition and preprocessing of data from a fast ADC. Other tasks, such as user dialogue and data file organization, could be carried out by a host computer with a suitable operating system for the time-shared operation with several users. The resulting organization is illustrated in Fig. 10.12a. The MASDAT-satellite processor has the following functions: control and timing of the ADCs and the spectrometer; acquisition of spectral signals; acquisition of additional slow signals (e.g. temperature and pressure of an evaporation, or a parallel GC detection); reduction of the digitized spectrum to 'peak candidates'; and transfer of data to the host computer. For each spectrometer, the host computer has the following functions: transformation of peak candidates into the final spectrum (MACO); generation of the interscan report; transfer of the final spectrum to disc

a)
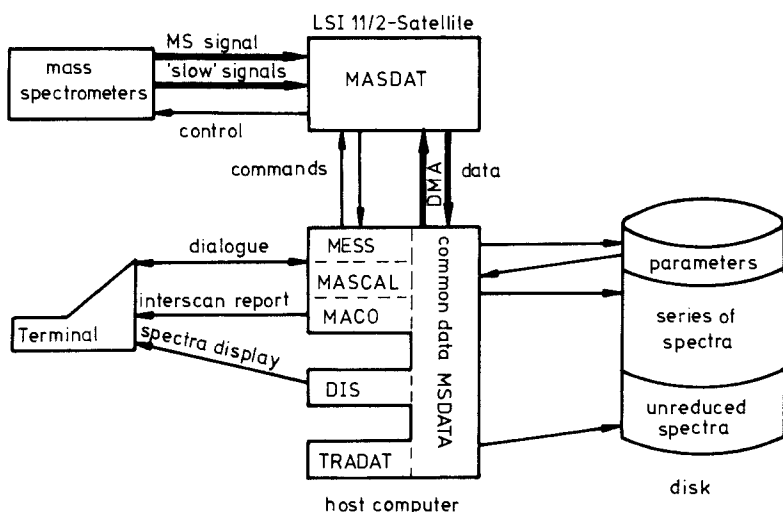


b)
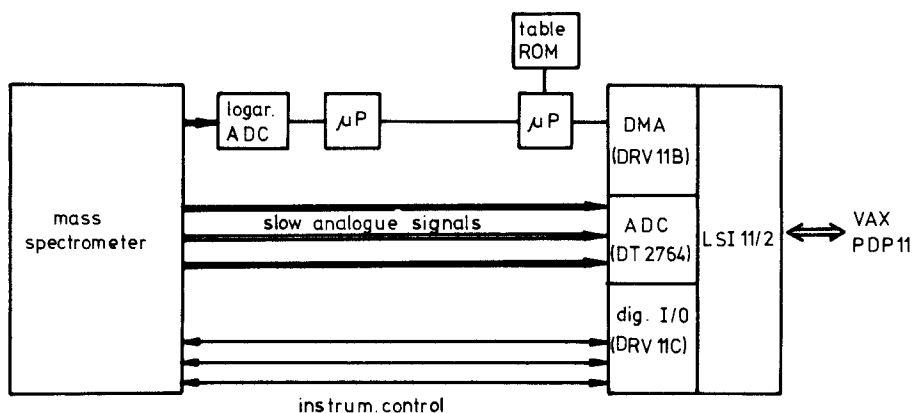


**Fig. 10.12** Computer system for measuring fast-scan, low-resolution mass spectra. (a) Organization of measuring spectra. The MASDAT satellite controls the instrument and samples the signals from the spectrometer. The host computer interacts with the operator (MESS), writes final spectra (MACO) or unreduced spectra (TRADAT) to external storage or performs a calibration (MASCAL). (b) Hardware configuration of the MASDAT satellite. The spectrum signal is sampled through a logarithmic A/D converter controlled by a microprocessor. Another microprocessor is used to convert the logarithmic A/D result. (Reproduced from [36] with permission of Elsevier).

(MACO); transfer of the unreduced spectrum (TRADAT); dialogue with the oper-
ator (MESS and MACO); real-time display of single spectra (DIS); and calibra-
tion of the mass scale (MASCAL). Hence, the MASDAT-satellite computer is
designed to interface with a single mass spectrometer; no mass storage peri-
pherals or user terminals are connected to it. The hardware configuration of
this computer is shown in Fig. 10.12b. The chief requirements of this data
system are as follows:

(a) The process of measuring spectra must be separated from all interpreta-
tive steps.

(b) All spectra must be measured in the repetitive scan mode.

(c) The computer system must not limit the capabilities of any instru-
ment.

(d) Three different operating modes should be possible: (1) to store nor-
mal spectra (mass/intensity pairs) only (normal operation); (2) to store the
complete analogue signal in digitized formed for diagnostic purposes or for
subsequent mass scale calibration; and (3) to save special investigations in
both forms in parallel.

(e) The calibration of the mass scale should be based on mass numbers be-
ing a function of time.

(f) The process mass scale calibration should be made as easy as possible
without being fully automatic: the operator should be able to make correc-
tions on the graphical display of certain parameters and should obtain informa-
tion on the state of the instrument (e.g. resolution).

(g) During the actual measurement of a series of spectra, an interscan
report should be produced, containing one line of information on each spec-
trum measured.

(h) The operator interface should allow for easy operation.

(i) The computer system should be capable of servicing more than one spec-
trometer at a time without limiting measurement conditions such as data rate,
scan time or cycle time.

## 10.3.2 Multiplex spectrometric methods

The simultaneous detection of optical signals at different wavelengths is
a very important advance in spectroscopic techniques. In addition to resulting
in a higher speed of acquisition of discriminated optical signals, it offers a
series of advantages, one of the most relevant of which is increased signal-
to-noise ratios. Inasmuch as the intrinsic features of spectroscopic tech-
niques are markedly different, multiplex methods cannot be applied to them in
a generic manner; each of the numerous alternatives described to date is
suited to a different extent to each spectroscopic configuration.

The word 'multiplex' has been used indiscriminately by many authors to describe both simultaneous and rapid sequential (e.g. rotating filters, fast-scan or slow-scan systems) multi-wavelength detection methods [37]. Other specialists use this designation to refer to spectroscopic methods based on the truly simultaneous measurement of several optical signals at different wavelengths. Finally, others reserve this term for those methods which use a single detector for multi-wavelength detection [17].

An essential feature of these multi-wavelength spectrometric methods is the generation of a large number of data at a high rate, which calls for the use of a computer for their acquisition, processing and delivery as required. Despite the different classifications of multiplex methods, only that based on the number of sensing elements used to perform multidetection will be used in their description here. In Fig. 10.13 is shown the classification proposed by Busch and Benton [37], which includes all the variants of atomic methods in atomic spectroscopy, although it offers an overview of general insterest. It should be borne in mind that the features and requirements of atomic spectroscopy differ significantly from those of molecular spectroscopy, basically because of the differences between atomic and molecular spectra. Thus, Fig. 10.13 is applicable to other types of spectroscopy only in an approximate manner.
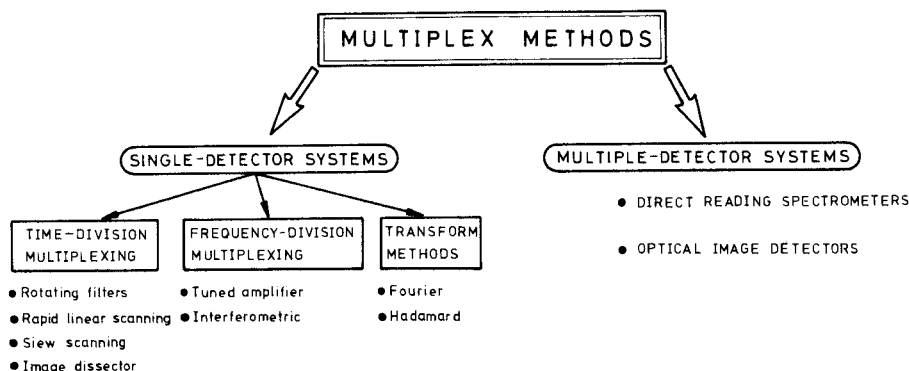


**Fig. 10.13** Multiplex approaches in atomic spectroscopy. (Adapted from [37], with permission of the American Chemical Society).
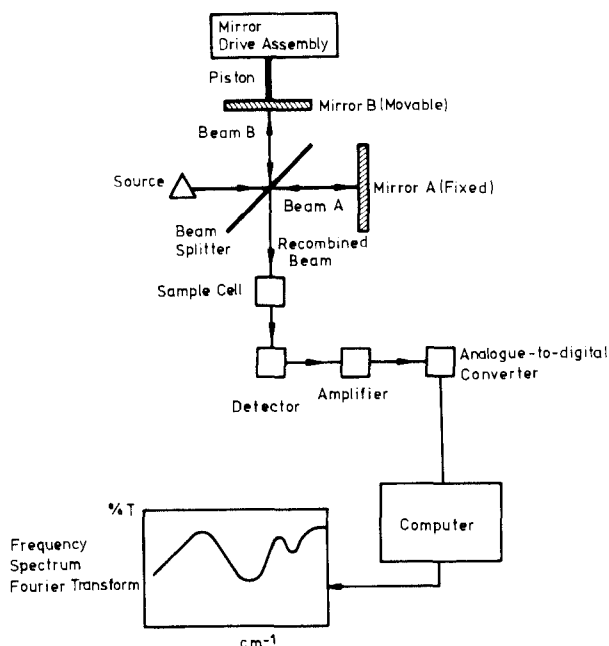
### *10.3.2.1 Multiplex methods using a single detector*

There are several possibilities for rapidly and sequentially arranging various optical wavelengths to strike a single detector in the so-called 'time-division multiplexing methods', which basically do not differ significantly from conventional spectroscopic methods except for the rate at which the optical signals are generated at different wavelengths. These methods roused some interest when they were introduced, but are clearly excelled at present by other alternatives.

In frequency-division multiplexing methods, more than one wavelength at a time is allowed to strike the detector. By modulating each wavelength of interest at a different frequency, wavelength discrimination can be accomplished electronically after detection by some means (e.g. by using separately tuned amplifiers to respond to the individual optical wavelengths). Modulating frequencies must be sufficiently different to avoid cross-talk between wavelength channels. This, together with the shot noise arising from the use of photomultiplier tubes, seriously limits the number of usable 'channels' [38].

Transform methods [39] are probably the alternative of greatest practical interest among multiplex methods using a single detector. Their intrinsic characteristics are better suited to IR [17,39] and Raman [40] spectroscopy and to NMR spectrometry [39]. It is of interest to note that their success in the UV-visible region with photomultiplier detection has been only marginal. The essential feature of transform methods is that the sample sees all the wavelengths in the region of interest at all times, instead of only a small portion at a time; this can result in significantly improved speed, resolution and signal-to-noise ratio over conventional dispersive spectroscopy. Because ordinary detectors (e.g. those sensitive to IR radiation) are unable to produce such a waveform directly as their response times are much too long, it is necessary to modulate the incoming stream in such a way as to convert the frequency of the incoming radiation to a value that can be followed by appropriate detectors. The Michelson interferometer (Fig. 10.14) is actually a device performing frequency-division multiplexing in that each wavelength in the spectrum is modulated at a characteristic frequency determined by the speed of the mirror. The complex pattern resulting when all of the modulation waveforms are simultaneously received by the single detector is called an interferogram [41]. To decode this time-domain spectrum into a conventional spectrum a complex mathematical treatment (Fourier transformation) and highspeed digital computers are required. Most commercial FT-IR instruments have built-in computers and there is no apparent difference from the classical manner of obtaining IR spectra.

**Fig. 10.14** Michelson interferometer and associated electronics. (Reproduced from [41] with permission of Allyn & Bacon, Inc.).


### 10.3.2.2 Multiplex methods using multiple detectors

These are also known as multi-channel spectroscopic methods and are based on a peculiar multiplexing mode denominated 'optical multiplexing' or 'wavelength-division multiplexing'. The detection system commonly used involves several independent detectors strategically located so that the dispersed radiation strikes each of them discriminately. The optical dispersion can be accomplished with the aid of a diffraction grating or prism, or both (échelle dispersion).

The simplest application of these multiplexing methods involves the socalled 'direct-reading spectrometer', which was used with some success for a short period in atomic spectroscopy [42]. This instrument consists of a dispersion system with an array of exit slits arranged at appropriate locations. Behind each slit is a photodetector —usually a photomultiplier. These multiplexing methods have also been used in UV-visible spectroscopy, although to a lesser extent: they have been implemented on automatic discrete analysers featuring an optical system of this type with 5-10 'channels' or wavelengths

which facilitate their automation. A representative example is the Hitachi 705 batch analyser, described in Chapter 8.

These systems have two major limitations: on the one hand, the number of channels must be small as each sensing element, however miniaturized, occupies considerable space with its independent associated electronics; on the other hand, and as a result of the first limitation, the spectral information obtained is necessarily partial, although in some cases (e.g. in atomic spectroscopy and routine UV-visible determinations) it is more than sufficient. Nevertheless, the development and consolidation of electronic image sensors have displaced them to a less prominent place in the context of simultaneous multi-detection.

One of the systems typically employed for the implementation of multiplexing spectroscopic methods is the microcomputerized configuration for processing data from a three-channel atomic absorption spectrometer reported by Adams *et al.* [43]. This *on-line* coupled system is depicted schematically in Fig. 14.15. The microcomputer (Apple II with 48 kb of RAM and a single 5.25-in floppy disc drive for program and temporary data storage) monitors the signal
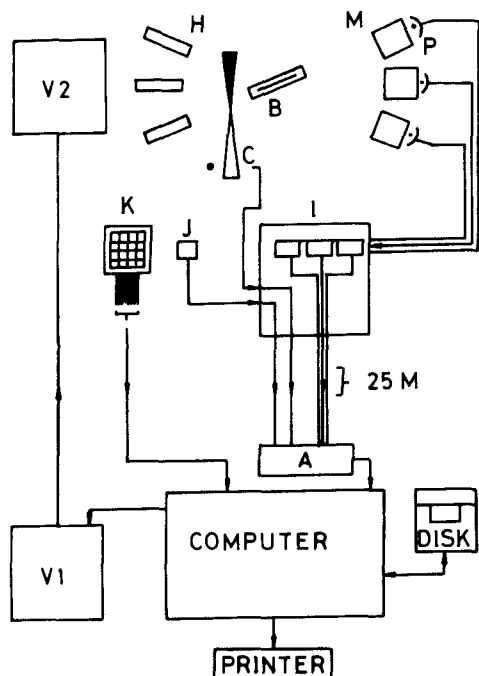


**Fig. 10.15** Three-channel AAS coupled with a microcomputer for processing of data. H: hollow-cathode sources; C: rotating sector; B: burner; M: monochromator units; P: detectors; J: beam chopper and start/reset unit; I: spectrometer control panel; A: analogue/digital converter; K; remote terminal; V: video monitors. (Reproduced from [43] with permission of Elsevier).

voltage lines from the detectors and the reset control and rotating chopper output reference signals, which are digitized with the aid of an 8-bit, medium-speed (5 kHz bandwidth) analogue-to-digital converter. A thermal printer is also connected to the system for output of the final results. The operator communicates with the computer via a microterminal and a video unit installed adjacent to the spectrometer. The system is employed for the routine determination of calcium and magnesium in soil extracts.

Multiplexing spectrometric methods with multi-channel image detectors make the most advantageous alternative to the simultaneous detection of optical signals at different wavelengths without the limitations of direct-reading spectrometers. There are a variety of optical image detectors: the photographic plate, the vidicon, the charge-coupled device, the charge-injection device and the linear-photodiode array (LPDA). The last, which is also the most interesting, combines three basic elements (radiation sensor, storage and readout system) into a single integrated circuit [16]. This multi-detector is a large- scale integrated circuit fabricated on a single monolithic silicon crystal. The device consists of an array of diodes (from 128 to 4096), also called 'elements', 'channels' and 'pixels', each acting as a light-to-charge transducer and a storage device. The LPDA is better suited to UV-visible spectrometry (molecular and atomic) than are most other image sensors because of its high quantum efficiency (about 40–80%), throughout the UV-VIS-nearIR range (190–1100 nm), large dynamic range and geometric, radiometric and electronic stability. The integrated circuit package also contains the necessary scanning circuitry for readout of the array. Address control circuitry is associated with each individual sensor or diode. A reverse-biased p-n junction diode serves as each photosensitive element, and addressing (readout) is effected by a shift register. Readout is accomplished by using two TTL level signals, a start pulse and a clock. Each photodiode is connected to the output line of a field effect transistor switch, which is controlled by a single bit that is shifted through the shift register. The circuitry can accomplish the recharging of each diode in less than 1 μs with the multiplexer switching between elements occurring at a clock rate of 250 kHz (4 μs per diode) to 2 MHz (0.5 μs per  diode). Once a scan has been initiated by a start pulse, the entire array must be scanned sequentially. The analogue signal from the common output line of the detector is run through an amplifier sample and hold system, which reduces noise; the signal is then digitized and transmitted via a suitable interface to a microcomputer. For many applications, continuous acquisition and storage of LPDA spectra is impractical as the number of spectra acquired quickly exceeds the computed memory. To prevent diode saturation during the interim period, the computer system periodically initiates a read

cycle of the array without digitization (periodic refreshing of spectral infor-
mation prior to spectral acquisition). One intrinsic advantage of LPDA is that
spectral data are directly available in digitized form for storage, retrieval
and software manipulations by a number of digital alogrithms such as log
(absorbance) for direct comparison with standards; arithmetic operations on
spectra; spectral derivatives both in the time and in the spectral domain for
enhanced qualitative characterization and resolution of spectral and chromato-
graphic peaks (see Chapter 13) and spectral deconvolution by the principles of
overdetermination and factor analysis. The LPDA detection of time-varying data
is especially suitable for chemometric techniques.

Currently most commercial UV-visible spectrophotometers have optical con-
figurations based on multi-detection with LPDAs. This is particularly the case
with continuous photometric HPLC and FIA detectors [44] although, in general,
almost every model offers appropriate software and hardware for adaptation as
a continuous detector. Most of these instruments have built-in microprocessors
of sufficient capacity for routine applications. Thus, the Hewlett-Packard
Model 8540A, which uses two photodiode arrays to allow the optical spectrum to
be scanned between 200 and 800 nm, includes a 16-bit computer with a large
variety of data-processing options. However, many users require data-process-
ing capabilities which are not included in the instrument as supplied. Pardue
*et al.* [45] designed an interface to connect this instrument to a general-pur-
pose computer, as well as a variety of software suitable for different appli-
cations. Some commercialy available LPDA instruments for both spectroscopic
and chromatographic applications suffer from very limited temporal and spec-
tral efficiency. For this reason, Rossi and Pardue [46] designed an inexpen-
sive fast memory module for rapid acquisition of digital data from a diode
array spectrophotometer. Figure 10.16 shows the block diagram of the elec-
tronic data system coupled with a previously described [4] optical system. The
detector is a solid-state photodiode array [Model 1412, Princeton Applied
Research (PAR)] containing 1024 pixels. Scan parameters such as the integra-
tion scan time, as well as data digitization, are handled by the detector con-
troller (Model 1218, PAR.). The detector scanning is initiated by the host
computer (Model PDP 12-30, Digital Equipment Co.) through a general-purpose
parallel interface, a network of encoding logic and a control bus multiplexer,
to the detector controller. Alternatively, the scanning can be initiated
through a switch register to allow spectral scans to be synchronized with the
start of experiments. The digitized data from the detector-controller are
directed along the data bus through a series of latches and drivers to the
memory module, where they are stored. A flag pulse from the detector con-
troller strobes each datum into memory. The data rate during a scan is 39.2
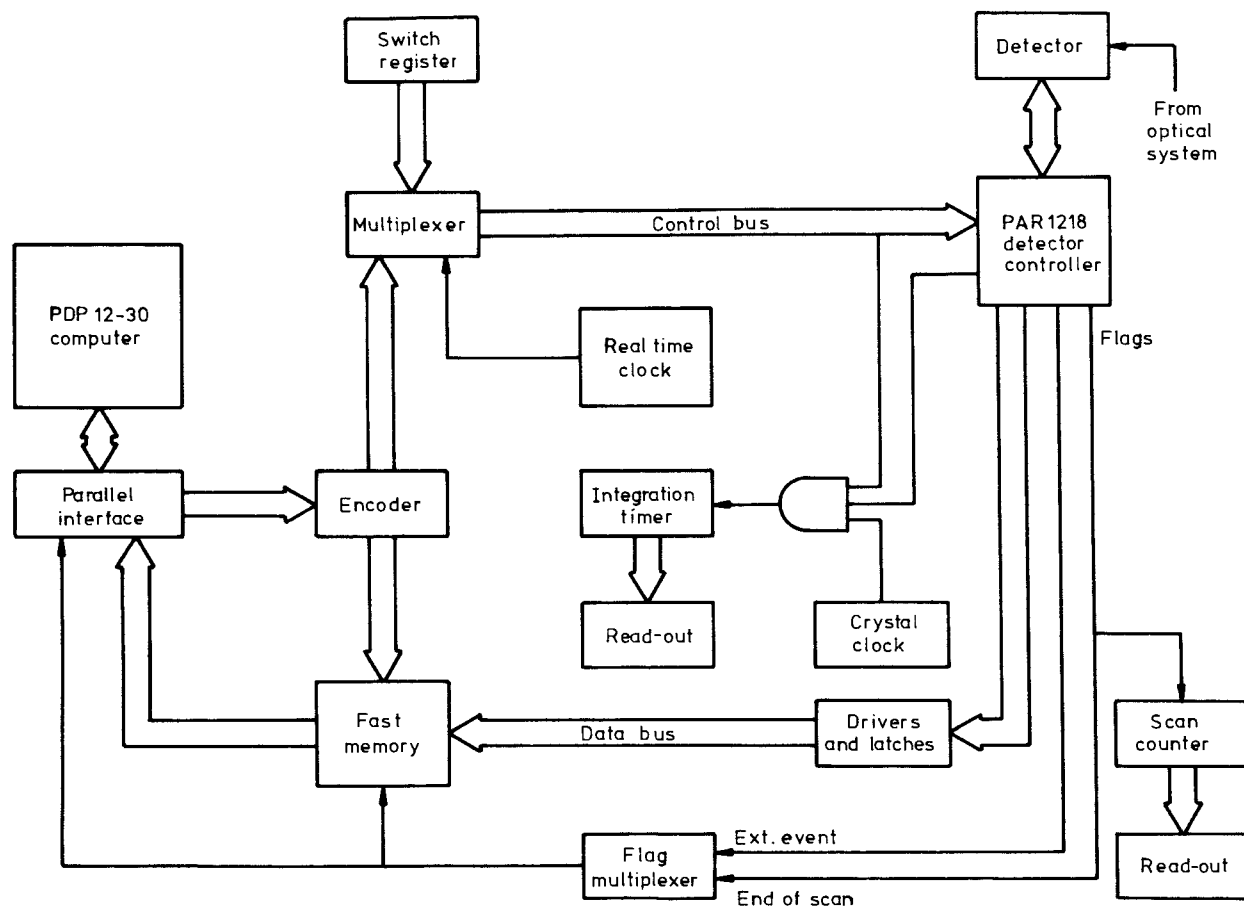
**Fig. 10.16** Data acquisition system and computer/operator interface for an LPDA spectrometer. (Reproduced from [42] with permission of Aris & Taylor, Ltd.).

kHz. A flag pulse, marking the end of a scan, is transmitted through the parallel interface to the computer, which therefore needs only to count the scans (40 Hz or less) and halt data collection when the desired number has been reached. The spectral data contained in the memory data are then transferred to the host computer's memory, under program control, for processing, bulk storage and/or readout.

LPDA UV-visible spectrometry offers more advantages and a wider scope of application that its conventional counterpart. A comprehensive description of this methodology is beyond the scope of this book. Representative examples of its applications are the study of the hydrolysis of penicillin [47], multi-component metal determinations [48] and the simultaneous determination of five clinically relevant haemoglobin derivatives in blood [49]. Multi-wavelength applications of derivative spectra obtained in LPDA spectrometers offer significant advantages, including improved signal-to-noise ratios via signal averaging and the ability to resolve mixtures with overlapping spectra. In addition, the matrix least-squares data-processing methods developed for absorption data can be incorporated as part of the software of commercial LPDA instruments and thus be directly applied to multi-wavelength first- and second-derivative spectra with success [50].

Despite the higher sensitivity and selectivity of molecular fluorimetry compared with UV-visible spectrophotometry and the remarkable development of high-performance conventional spectrofluorimeters in the past decade, it is interesting to note the scarce development of instruments with image detectors for fluorimetric measurements in contrast with the increasing availability of commercial LPDA spectrophotometers. One of the more commonly used image detectors is the vidicon [51,52], which, with the aid of suitable software, offers three-dimensional plots of great use [34], particularly when appropriate mathematical calculations are applied through a computer for quantifying chemical compositions [55]. The rapid scanning capability of the video fluorimeter [54,55] to acquire emission-excitation matrices (EEM) has been applied [56] to the phosphorimetric determination of mixtures of polynuclear aromatic compounds; this alternative overcomes the problem of the convolution of time decay with phosphorescence excitation and emission spectra by integrating the signal on a target. This method also circumvents the need for very rapid acquisition of data from samples with phosphors of very short lifetimes. A phosphorescence emission-excitation matrix (PEEM) obtained in this manner allows time resolution. Sets of each time-PEEM allow a ratio deconvolution algorithm to successfully resolve mixtures of analytes. Thompson and Pardue [57] reported in 1983 the measurement of synchronous fluorimetric spectra by use of a silicon-intensified target vidicon, thus replacing the conventional

mechanically scanned mode by the electrically scanned imaging detectors, which afford substantially improved versatility and rapidity, particularly in the optimization of instrumental parameters, over the conventional mode, which is cumbersome and time-consuming. There are few LPDA spectrofluorimeters, a representative example being the microprocessor-controlled multi-channel fluorimeter designed by Oldham *et al.* [58] for the determination of chlorophyll a in sea water. It is a portable instrument for continuous use on board a ship, which requires a high degree of automation in addition to rapid data acquisition and treatment capabilities in order to provide the spectral information with sufficient time resolution to allow topographical mapping. The detection system used is a Tracor Northern IDARSS system, consisting of an intensified 512-element array. A circular variable filter wheel provides the excitation resolution, the wheel being driven by a 200-step stepping motor. Both the diode array and the stepping motor are interfaced to an Apple II+ computer with 4 kb (8 bit/byte) memory, two floppy disc drives and a CRT monitor. An Apple Super Serial interface provides the RS-232C serial communication link between the computer and the multi-channel analyser which controls the diode array. The stepping motor is accessed by a controller via a parallel interface.

Atomic spectroscopy has used image detectors since the early 1970s for the multi-determination of metal species by taking advantage of the scarcity of spectral interferences in comparison with molecular spectroscopy [37,59]. Spectrometer systems incorporating LPDA as multi-channel detectors for atomic emission [60,61] or absorption [62,63] have been described. A straightforward computerized calculation procedure affording a major enhancement of wavelength measurement accuracy through the use of LPDAs as a detectors for ICP-AES has been reported [64]. The use of LPDAs as detectors for simultaneous molecular and atomic spectrometric measurements with electrothermal atomization lowers the sensitivity and spectral resolution of atomic measurements, but offers two advantages of great interest, as it makes possible the simultaneous determination of a large number of elements and facilitates the study of chemical interferences and molecular evolution at temperatures unattainable by the devices typically used in conventional UV spectrometry [64]. Two-dimension multi-channel detectors such as the vidicon have also been used in atomic spectrometry [38]. Olesik and Hieftje [66] evaluated two optical systems for the acquisition of two-dimensional images for spatial mapping of inductively coupled plasmas and flames in atomic spectrometry; a silicon intensified target vidicon detector provided quantitative images in time-integrated or time-resolved modes by means of a data acquisition control system. Hulmston *et al.* made a critical comparison of two types of multi-detection not based on image

detectors, namely spectrographic recording and spectrometric recording by means of a rapid scanning monochromator, for multi-element analysis by ICP [67]. Both optical systems were computer-controlled.

## 10.4 SPECTROSCOPIC DATA BANKS

The interpretation of spectroscopic data for the identification and structure elucidation of organic compounds is largely an empirical process and relies heavily on the use of previously accumulated reference data. Compilation of computer-readable spectroscopic data bases is nowadays feasible because most commercially available spectrometers have small built-in computers for the digital acquisition of measured spectroscopic data; they are also equipped with a suitable mass storage device to store spectra or selected spectral data, or they provide the facility to transfer the recorded spectra to a more powerful external computer. If the computer-readable spectroscopic data are suitably organized, the analyst is provided with a very powerful tool for the identification of a compound, a group of compounds or a structure by means of suitable software, thereby avoiding the slow and tedious manual work otherwise involved [67,69].

For over a decade, a number of research teams have pursued the automation of this last, interpretative stage of the analytical spectroscopic process. There are two general ways of approaching this problem: by using library searching systems or artificial intelligence systems (pattern recognition and expert systems) which are commented on below.

### 10.4.1 Spectral library searching systems

This is the earlier of the two ways of automating the interpretative stage [70]. This procedure looks for identity or similarity between the spectrum of an unknown substance and one or more spectra from a standard collection stored in the computer. As a result of the search, the computer outputs a list —as short as possible— according to *a priori* fixed criteria of similarity [71]. The library-search algorithms supply two types of results according to the degree of success: (a) when the unkown spectrum has an exact match within the spectra stored in the data base, one obtains an exact determination of the unknown structure, i.e. with a high degree of probability; (b) when the unknown spectrum does not have an exact match in the collection of reference spectra, one obtains a 'hit-list' of 10-15 compounds whose spectra are sufficiently similar to the unknown and, thus, information about the chemical class of the molecule and/or substructures contained in it —this is the commoner situation.

In spite of the commercial availability of hardware and software for spectral library searching, particularly in IR spectroscopy, the procedure suffers from major shortcomings such as the lack of reliability in the identification and/or elucidation, the need for computers with large memory capacities, the high cost of software storing the collection of standard spectra (from 5000 to 200 000) and the unfeasibility of obtaining collections containing over 1% of all known organic compounds. Moreover, prestigious authors in this field recently acknowledged [71,72] having reached a level from which significant advances are difficult to achieve by simply comparing spectra.

## 10.4.2 Artificial Intelligence systems

To circumvent the above-mentioned shortcomings one may opt for automation of the interpretation of spectra, generally through the identification of substructures of the unknown compound by using statistically sound correlation tables which take up much less memory than a wide collection of spectra. In other words, the aim is to store the maximum possible chemical information extracted from each spectrum in the computer's memory in order to avoid unnecessary repetitions. These procedures are intended to replace to a greater or lesser extent the process taking place in the human brain in the empirical interpretation of spectra. Efforts to develop computer programs for this purpose have met with varying degrees of success. These artificial intelligence systems have two general approaches, namely pattern recognition and expert systems.

### 10.4.2.1 Pattern recognition

This is a branch of artificial intelligence (AI) which tries to find a relationship between a characteristic set of features (e.g. a spectrum) of an object (e.g. a compound) and some property (e.g. the presence of a particular functional group) without requiring chemical knowledge or imposing chemical prejudices. To predict the presence or absence of a functional group in the unknown compound, a relationship must be established between the presence of that functionality and the expected pattern in the spectrum. This relationship is established through a procedure called 'training', and a training set of identified spectra is needed. The success of this approach is heavily dependent on the availability of a representative high-quality training set. As it is expensive to accumulate such a data base, very few throrough accumulations have been established. Pattern recognition studies of structure elucidation attempt to find what amounts to interpretation rules by using numerical methods rather than human intuition [73]. A variety of algorithms are used for this purpose. One of them involves the generation of a data matrix from the IR

spectrum of the unknown substance, which are then sorted out and normalized. The normalized IR matrix is interpreted by means of an algorithm which allows the recognition of up to 32 substructures [74]. An interesting approach in this respect involves the hierarchical organization of the data base, after a hierarchical tree has been generated [74,76].

### 10.4.2.2 Expert systems

This approach, currently rousing the interest of an increasing number of workers, uses a completely different data-structure presentation in the data-based computer system. This new type of spectroscopic data base contains not only purely spectral data (factual knowledge), but also —and in many cases, only— information and rules (procedural knowledge). In addition to the novel type of data base, new problem-solving algorithms (for structure identification) that embody and reflect the knowledge, experience and intuition of the expert spectroscopist have been elaborated, creating so-called 'expert systems' for problem solving in structure elucidation [72].

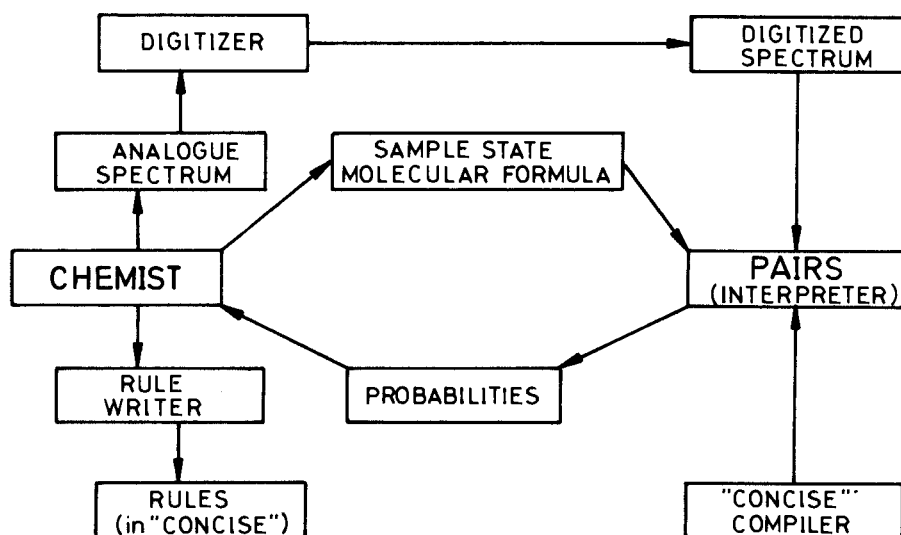An expert system for structure elucidation consists of three main parts:

(a) The knowledge base, which, in addition to a collection of spectra, contains properly structured knowledge obtained from a human expert. This procedural knowledge is usually stored in the form of production rules, which are connections between two assertions specifying how the presence (or absence) of a piece of evidence affects the truth of a hypothesis. In structure elucidation, such production rules may be a set of well-defined spectrum-structure correlations.

(b) The inference engine, which is a module (also separated) of utility programs that infer suggested solutions from the facts stored in the data base. Much more sophisticated and powerful algorithms than simple library-search are used for structure elucidation [77].

(c) The user's interface, which ensures smooth communication with the system, enabling changes of its method of working and sometimes makes it possible to explain the suggestions provided or to draw some novel conclusions while the system is running.

A characteristic example of the earliest approaches to the use of expert systems for structure elucidation is the program PAIRS (Program for the Analysis of IR Spectra) [78,79]. Figure 10.17 shows the information flow in this expert system. The production rules are written in an English language called CONCISE (Computer Oriented Notation Concerning Infrared Spectral Evaluation) and are separated from the interpreter of the inference engine. Another similar program for data from a single spectrometric technique is the DENDRAL, designed for mass spectrometry [80].

**Fig. 10.17** Expert system for elucidation of chemical structures (IR spectroscopy). (Reproduced from [78] with permission of the American Chemical Society).

Every spectroscopist is aware of the fact that spectral data from a single technique do not contain sufficient information to elucidate with high precision and accuracy the structure of an organic compound. Frequently, data from two or more spectroscopic techniques are integrated to improved the safety level in the elucidation of chemical structures as the diverse nature of the data results in a positive synergistic effect upon integration. There are several computer programs which allow the automatic identification/elucidation of structures: CASE [81], STREC [82] and SEAC [83]. Nevertheless, these programs are of the 'old fashioned' type because updating of the knowledge (mostly buried with the code) is achievable only by painstaking reprogramming or even by reconstruction of the basic architecture of the system. An entirely novel approach was developed more recently [72,84]. It involves a set of carefully designed expert systems in which all knowledge data bases are detached from the program modules (inference engines) and a very flexible, clear and consistent user's interface. This approach is called IIAI (Independent Identification by Artificial Intelligence).

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# 11

## Automation of analytical instrumentation. II. Electroanalytical techniques

### 11.1 INTRODUCTION

Electrochemical analysis is composed of a vast range of techniques such as potentiometry, polarography, amperometry and coulometry. Selection of the technique best suited to the purpose and of the appropriate working conditions ensure a high degree of sensitivity and selectivity.

Although optical techniques —particularly photometry— prevailed in automatic methods of analysis for a long period, the advent of ion-selective electrodes (ISEs) marked the beginning of the automation of electroanalytical techniques. The variety of analysers currently available that incorporate electroanalytical detection not only outperform those based on optical sensing (e.g. in analyses for alkali and alkaline-earth metals with ISEs as opposed to flame photometry), but also they have fostered the development of *in vivo* measurements, no doubt the most exciting and promising area of clinical chemistry.

The advantages of electroanalytical detection over other types of sensing can be summarized as follows:

(a) It has a broad scope of application covering both organic and inorganic substances.

(b) It allows analyses to be carried out in a variety of media (e.g. aqueous, gaseous, molten salts).

(c) It is not influenced by the coloration or fluorescence of the sample.

(d) It is sensitive to the activity of dissolved species (e.g. ISEs), a feature of great interest in clinical chemistry.

(e) It is affordable. As a rule, electroanalytical instruments, with the exception of a few sophisticated, brilliant performers, are cheaper than their optical counterparts.

Obviously, electroanalytical techniques possess some disadvantages such as electrode poisoning, particle deposition on the sensing surface and problems arising from the chemical irreversibility of some processes, all of which require a careful choice of the particular technique to be applied.

Not all instrumental techniques lend themselves so readily to computeriza-

tion. In this respect, electroanalytical techniques use current/potential per-
turbations and the responses obtained are also analysed in electrical terms
(E, i and q), so they are particularly suitable for computer control [1],
which offers a number of advantages, namely:

(a) Ready generation of complex exciting waveforms that are difficult to
obtain by means of analogue systems.

(b) Easy modificaction or incorporation of different techniques which can
be implemented by simply re-adapting the available software to the pursued
end. Conversely, analogue systems require major alterations to the system's
circuitry.

(c) Convenient data acquisition and treatment. Microcomputer-controlled
systems allow the acquisition of a large number of data in a short time and
their simultaneous or sequential treatment —analogue systems only afford se-
quential treatment. This allows the acquisition of increased signal-to-noise
ratios by digital filtering of the response signal and the elimination of unde-
sirable phenomena such as the ohmic drop of the electrolytes used as conduc-
tors in electrochemical cells.

(d) Reduction of the mechanical systems involved (e.g. through the elimina-
tion of the mechanical control of the drop time in liquid electrodes).

(e) Cost reduction. The great versatility of electrochemical systems re-
sults in greater instrumental simplicity and hence decreased costs.

All these advantages have fostered the automation of electroanalytical
techniques to such an extent that they have equalled and even excelled the suc-
cess achieved by other analytical techniques. Their automation has been propi-
tiated by the serious technical difficulties involved in some instances (e.g.
potentiometry) or imposed by the requirements of adequate performance (e.g.
voltammetry).

The recent literature on electroanalytical techniques almost invariably
features the use of microcomputers. Advances in this field parallel the
incorporation of the latest electronics and the use of digital circuits to
perform functions formerly carried out by analogue systems. Indeed, the great-
est breakthrough in this respect has been the development of integrated cir-
cuit microprocessors, whose current affordability has fostered their massive
incorporation into analytical instrumentation. The added affordability of inte-
grated-circuit memories and analogue-to-digital (A/D) and digital-to-analogue
(D/A) converters has facilitated the development of inexpensive instruments
that permit closed-loop control of data acquisition and processing.

This chapter deals with the latest developments and state of the art in
the automation of electroanalytical instrumentation as a logical result of the
intrinsic characteristics of each technique and the potential offered by tech-

nological developments to scientists capable of joining them in a brilliant, effective manner.


## 11.2 AUTOMATION IN POTENTIOMETRY

Potentiometry is one of the most readily automatable electroanalytical techniques. Hence the first few steps in the automation of potentiometric measurements were taken over 50 years ago with the earliest electronic volt-meters. The replacement of compensating bridges by electronic instruments sub-stantially reduces the measurement time in potentiometric analysis. In addi-tion, because of its high input resitance, the voltmeter reduces the errors arising from the polarizing current flowing in the circuit when the bridge is off-balance.

The selection of the appropriate time for carrying out readings of the cell potential is a key aspect of automated potentiometric measurements. If the time required for equilibrium to be reached is too long, it may result in further problems arising from drifts in the electrode potential (e.g. changes in the solution composition resulting from contact with the electrode or changes in the salt bridge or the reference electrode). The automatic selec-tion of the reading time in potentiometric measurements is an excellent solu-tion to these problems. The principle behind this selection varies from instru-ment to instrument. In some instances, readings are made at fixed times from the immersion of the electrode in the unknown solution, which is a serious drawback. Insofar as the response time of ion-selective electrodes (ISEs) depends on their service time, it is preferable to monitor the time-dependence of the electrode response by means of a suitable electronic circuit and select the reading time according to it. In other instruments, readings are made imme-diately after the rate of potential change has fallen below a preset value.

In dealing with the automation of potentiometric measurements a distinc-tion is made below between 'commercially available' and 'home-made' instru-ments.


### 11.2.1 Commercially available instruments

There is a wide range of automatic potentiometric instruments available from different manufacturers, ranging from straightforward pH-meters to sophis-ticated multi-parameter analysers. The great development of ISEs has contrib-uted to a significant expansion of this area with the advent of the so-called 'pX-meters', *viz.* instruments providing the negative logarithm of the analyte activity from potentiometric measurements. Every pX-meter should be equipped with adjustment of the slope factor over a wide range because the slope of the

calibration graph of ISEs may vary enormously depending on the ion charge and the electrode characteristics. The similarity between pH- and pX-meters is obvious, their chief differences being the pointer scale and the wide adjustment range of the latter.

The pH-meter is probably the most frequently used analytical instrument. It has undergone a series of improvements resulting in a high degree of automation. Former analogue pH-meters showed the reading through a needle moving on a scale; the latest digital pH-meters display results via a digital counter or a printer. pH-meters can also be classified according to whether they provide direct readings (i.e. they offer the result by means of a meter or with digits) or compensate the signal from the cell with another generated by the instrument (zero-setting instrument). Commercially available pH-meters commonly use direct readings which are displayed digitally. Potentiometric systems carrying out complex measurements use combined pH-meter/multiplexer units involving the measurement of signals from several measuring cells according to a preset sequence or a special timing program. The reduction of microprocessor costs has fostered their massive incorporation into straightforward laboratory equipment, of which potentiometric instruments are no exception. The enhanced performance and simple operation of such instruments and their reduced costs have considerably increased their popularity.

The Corning Ion-meter 135 and the Orion 811 are representative examples of pH-meters with and without a built-in microprocessor, respectively. The latter shows the cell voltage at equilibrium. Its temperature sensor automatically corrects the error due to the difference in temperature between the standards and the sample. When the potential difference between two standards varies significantly from the theoretical expectation, the instrument shows the deviation. The microprocessor of the Ion-meter 135 allows the automatic use of different measurement programs (e.g. standard additions, sample addition, pH determination). Data can be stored in the memory for subsequent calculations. The microprocessor can also be used independently.

To follow the trend of the automation of the potentiometric technique, various manufacturers have launched a series of single- and multi-parameter analysers for the determination of gases and liquids, which are discussed below.

The Radiometer ICA 113 is an improved version of their former ICA 1. It is a modular instrument for the automatic anaerobic measurement of calcium and pH in serum and plasma. Because of the close relationship between these two parameters, their simultaneous measurement is essential as a normal ionized calcium value may be accompanied by an anomalous pH and vice versa —this may lead to erroneous diagnoses. The sensor of the ICA 113 consists of an F2111 Ca Selectrode™ whose PVC membrane is protected from contamination by proteins by a

readily changed Cellophane membrane and a glass and a calomel electrode. Ionized calcium is measured at a standard pH of 7.40. All results are displayed or transferred to an external printer or computer via the built-in RS-232C interface. Before each measurement, the sample is automatically equilibrated with a gas mixture containing 5.7% $CO_2$ to compensate for the loss of carbon dioxide from aerobic measurements and return the pH to about 7.4. Up to 14 samples can be handled serially by the measurement tray; a complete cycle including equilibration, measurement and rinsing takes 83 min. A batch sample in progress can be interrupted at any time for STAT measurements on whole blood samples (25 µL). The computer controls all the vital parts of the system and displays errors, if any, on a screen.

The automated ISE-based (glass and valinomycin) instruments commercially available for the determination of sodium and potassium compete advantageously with flame photometers for the same purpose. Representative examples of this type of potentiometric instrument are the KNA1 sodium-potassium analyser from Radiometer, the Electrolyte II (in semi- and fully automatic versions) from Beckman and the Nova 1 from Biomedical Laboratories.

The relevance to clinical chemistry of instruments capable of determining gases or performing acid-base equilibrations on blood is obvious. Manufacturers have marketed various blood gas analysers for the determination of crucial parameters in human serum.

The latest acid-base analysers launched by Radiometer are intended for the automatic determination of various parameters in blood. Thus, Models ABL 30, 3, 300 and 4 determine pH, $pCO_2$ and $pO_2$. In addition, the ABL 3 and 300 determine haemoglobin (Hb) and the ABL 4 Hb plus K. All four analysers are based on the use of membrane electrodes and allow the measurement of the above-mentioned parameters on relatively low sample volumes (50-120 µL). Both the direct measurements (Hb, pH, $pCO_2$, $pO_2$ and potassium), and hydrogen carbonate in plasma, total $CO_2$, actual base excess, standard base excess, standard hydrogen carbonate, oxygen saturation and oxygen content are displayed and printed. Similar to these Radiometer models are the E4A and Nova 5, manufactured by Beckman and Biomedical Laboratories, respectively, and capable of determining Na, K, Cl and $CO_2$. The functions of both instruments are controlled by a microcomputer which performs three essential tasks: calibration with two standards per measured parameter, analysis and cell washing.

*In vivo* measurements, of great significance to the diagnostics of some diseases, have also been tackled with the aid of automatic instruments based on the use of membrane electrodes. Such is the case with the Radiometer TCM 20 transcutaneous analyser, a portable, battery-operated instrument allowing the continuous non-invasive monitoring of $pO_2$ and $pCO_2$. To achieve optimum perfor-

mace through the skin, the electrode system is composed of two pieces: a sturdy electrode body with a cable to the monitor and a highly flexible attachment ring. The liquid contact is applied after attaching the ring to the skin to ensure proper adhesion (the electrolytes in contact with the skin are neither toxic nor noxious). A monitor self-check procedure started automatically at regular intervals ensures smooth operation.

### 11.2.2 Home-made instruments

There is a wealth of literature on this type of instrument dealing with various aspects of direct potentiometric measurements.

The characteristics of ISEs were studied with the aid of a microcomputer-controlled potentiometric system allowing the convenient and rapid determination of the detection limit and coefficient of selectivity of the sensor in question. The system consisted of the following commercially available units: (a) an Altair 8800B microcomputer equipped with 32Kb RAM and the required peripherals (CRT, teletypewriter and cassette recorder); (b) a digital Orion 801 pH/pV-meter; and (c) a multi-speed burette governed by a SargentWelch S11120-12 step motor. The timing circuit of the pH/pV-meter was modified by changing the lower value components by a resistor and a capacitor in order to obtain a time display range of 0.09–0.6 s instead of the original 0.6–5 s. The internal square-wave clock of the burette controlling the speed of delivery was switched off and the pulses generated by the computer software via an opto-isolator circuit were fed directly to a step motor, thereby allowing direct control of the burette by the microcomputer. Home-made parallel interface circuits were used to transfer potentials from the BCD output of the digital pH/pV-meter to the microcomputer. A BASIC control program and double-precision arithmetics were also used. The experimental data, *viz.* $E_i$ and $a_{A,i}$ for evaluation of the detection limit and $E_i$, $a_{A,i}$ and $a_{B,i}$ (measured potential and activity of the primary and secondary ion, respectively) were subjected to non-linear least-squares regression. The parameters measured on three commercial liquid-membrane electrodes (chloride, nitrate and calcium) were consistent with literature values [3].

Home-made computer-controlled pH-stats developed so far can be classified into two categories: (a) those where the pH is kept by means of a delivery system into which the acidic or basic solution is introduced; and (b) those using a programmable coulostat to generate the protons of hydroxyl ions required for stable pH control.

Lemke and Hieftje [4] developed a computer-controlled pH-stat (Fig. 11.1) in which the reagent delivery system is based on a droplet generator, also computer-controlled, capable of selectively adding 0.06-µL aliquots. This precise

control of the reagent addition allows the pH to be adjusted to within 0.0008 units. The program controlling the instrument was written in FORTRAN IV. The pH-stat was operated by means of simple directives entered into an alteration of experimental parameters. To evaluate the pH-stat, standard calibration graphs were obtained from which the concentration of glucose in a sample of standard human blood serum was determined to within 3% of the value specified by the manufacturer. Two alternative measurement approaches, the fixed-time technique and the use of enzymatic induction times, were evaluated for the determination of glucose concentrations.
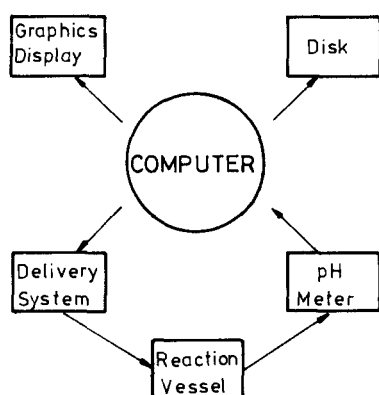


**Fig. 11.1** Block diagram of pH-stat with reagent delivery system. (Reproduced from [4] with permission of Elsevier).

A shortcoming of the previous pH-stat was the solution volume to be controlled increased indefinitely, as did the ionic strength. This affected the responses of the sensors. This drawback was overcome by using a microprocessor-controlled coulometric system. The instrument developed by Bergveld *et al.* [5] consisted of a coulometric pH system using a programmable coulostat to control the pH of a given volume of unbuffered solution. The scheme of the instrument and the instrumental assembly is depicted in Fig. 11.2. To maintain the current source floating, the coulostat is connected to a suitable output by means of two microprocessor-actuated opto-couplers. The required coulostat output is obtained by using a constant, pre-programmed value of the current output over a given time which is determined by the microprocessor and suited to the selected control program. The direction of the current is also controlled by the microprocessor, which triggers one or the other opto-coupler according to whether the current is positive or negative. The total control of the pH of

the test solution is accomplished by measuring it and comparing the measured value with the required value. Obviously, this pH measurement determines the precision of the pH control. Of the different electrodes assayed, ISFETs turned out to be the optimum pH sensors.
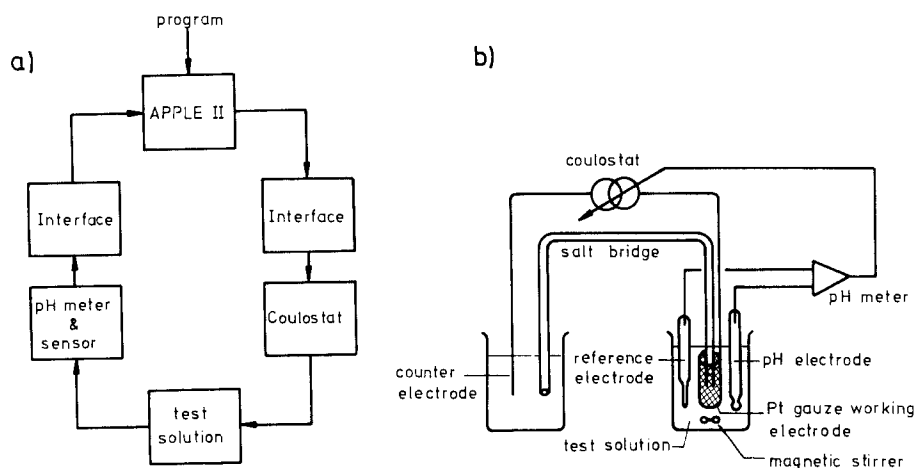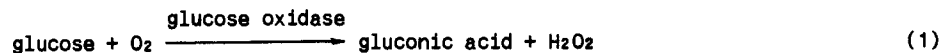


**Fig. 11.2** Coulometric pH-stat system. (a) Block diagram. (b) Experimental setup of the coulometric control system. (Reproduced from [5] with permission of Elsevier).

Figure 11.3 depicts the cell used by the automatic potentiometric instrument devised by Malmstadt and Pardue [6] for the enzymatic determination of glucose based on reaction-rate measurements. The chemistry involved is

$$\text{glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2 \qquad (1)$$

$$H_2O_2 + 2H^+ + 2I^- \xrightarrow{\text{Mo(VI)}} I_2 + 2H_2O \qquad (2)$$

of which reaction (1) is the rate-determing step. This concentration cell consists of a beaker containing the reference solution and a sample tube. Into both solutions are introduced similar electrodes sensitive to the same species (iodide), the concentration of which is kept constant in the reference com-

partment and continuously changes in that of the sample, which results in an emf change proportional to the rate of disappearance of iodide from the solution. This, in turn is proportional to the concentration of the sought-for constituents. The reagent and the sample are mixed in the concentration cell compartment. The solution is rapidly stirred and the emf changes recorded as a function of the reaction rate are fed to a readout device. The control system regulates sample and reagent addition, mixing and flushing of the system after measurements. The stirring unit effecting the mixing of solutions in the reference compartment ensures a stable and reproducible potential in the reference electrode. Temperature control is indispensable to keep the concentration cell, reagents and sample at a temperature constant to within $\pm 0.1^\circ$C. The system allows the determination of glucose concentrations between 5 and 500 ppm in a volume of 2 mL with relative errors of less than 1% and at a rate of 60 samples/h.
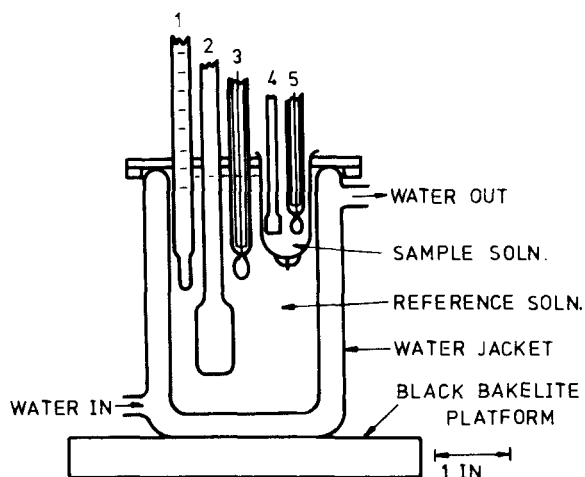


**Fig. 11.3** Concentration cell for the kinetic enzymatic determination of glucose. (1) Thermometer; (2) reference stirring rod; (3) reference electrode; (4) sample stirring rod; (5) sample electrode. (Reproduced from [6] with permission of the American Chemical Society).

Schwing *et al.* [7] developed a potentiometric-voltammetric system with digital data acquisition and treatment for the kinetic determination of mixtures of metal ions based on ligand-exchange reactions. The results obtained from potentiometric measurements are more precise than those found with amperometric measurements (the typical relative errors are 5% and 5-10%, respectively).

The computer-controlled direct potentiometric analysis system devised by

Ariano and Gutknecht [8] uses a small digital computer for real-time control of the instrument. A typical analysis involves a series of standard additions to the unknown solution placed in the cell containing the working (ISE) and reference electrodes (SCE). These additions are optimized in such a way that each volume yields an even distribution of the resulting cell voltage. This voltage, acquired by a computer-optimized sampling technique, and the standard-addition data are fitted to the Nernst equation by a non-linear least-squares procedure. The system has been used by their proponents to automatically analyse for $K^+$ over the concentration range $10^{-1}-10^{-3}M$ with an accuracy and precision of about 2%.

Sekerka and Lechner reported in 1973 an automated instrument later modified for various determinations. The original version [9] was conceived for the determination of low concentrations of fluoride and consisted of an $F^-$-selective electrode, a printer and a digital pH/pV-meter —all marketed by Orion— plus a peristaltic pump, a thermostating electrode elevator turntable unit and a home-made control unit. The functions of the system were as follows: a TISAB solution was added to the samples located on a holder, after which sample and reagent were mixed and the electrode was submerged in the vessel, which also acted as a measuring cell. After a programmable time, the cel voltage was read and printed. Then the electrode was thoroughly washed and made ready for the next analysis. The control module allowed flexible measurements and programming. The absence of feed-back between the detector and the control module imposed long analysis times (up to 10 min) to ensure acceptable precision in the case of very dilute samples (concentrations below $10^{-6}M$). The stabilization of the voltage cell was not checked at any time. A later version of this instrument [10-12] incorporated a minicomputer and an interfaced printer (Fig. 11.4). In addition to the above-described functions, the new system afforded conductivity and pH measurements and was chiefly applied to the determination of water hardness by the standard-addition method, combined with a subsequent 1:1 dilution and a potential measurement. The hardness due to carbonate was calculated by the computer from the equation

$$C = C_s \cdot \text{antilog} \left[ (E_0 - E_1)/29.5 \right]$$

where $C_s$ is the concentration (in ppm) of the $CaCO_3$ standard used, $E_0$ the electrode potential measured in the standard solution and $E_1$ the sample potential (both in mV). The total hardness was calculated from

$$C_T = C_A \cdot \text{antilog} \left[ \frac{\Delta E \cdot \log 2}{E_2 - E_3} \right] - 1$$

where $C_A$ is the concentration of the $CaCO_3$ standard, $E_3$ the potential measured after 1:1 dilution and $\Delta E=E_2-E_1$. Measurements were controlled by a separate module rather than the computer and there was no feed-back between the measuring and the control units. The analysis rate was 20 samples/h.
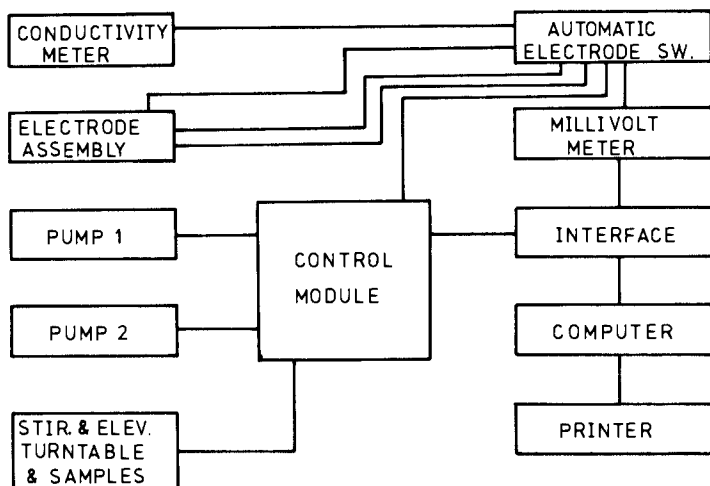


**Fig. 11.4** Scheme of the instrument designed by Sekerka and Lechner for the determination of water hardness. (Reproduced from [12] with permission of Marcel Dekker Inc.).

The Gran method has been succesfully applied both to the evaluation of calibration graphs and to standard addition and subtraction measurements. When large numbers of data pairs are involved, work is substantially simplified by the incorporation of a small computer in the measuring instrument. Frazer and co-workers [13,14] developed a method for the evaluation of titration or standard-addition curves. The so-called 'error function left' (EFL) method allows the selection of the linear portion of the Gran function yielding the best fit of the behaviour of the measuring cell to theoretical expectations. The method involves selecting the portion of the function where linearity is to be tested. This is accomplished by choosing an adequate slit width which is scanned over the assumed linear range in steps ensuring adequate overlap of data fields. Each data field yields a curve by linear regression, and each of the curves intercepts the axis corresponding to the standard or reagent volume. The EFL relationship used to simplify the calculations is obtained by plotting the differences between consecutive intercept volumes as a function of the volume of reagent added. The minimum of this plot corresponds to the theoretically linear portion of the Gran transformation, provided that an appropriate slit width and step were chosen. The application of the method to

a variety of problems proved its suitability for automatic potentiometric systems. However, the transformations involved are time-consuming unless a computer is used on-line. The determinations carried out by this method are chloride in water without [15] and with [16] standard additions, phosphate in the presence of high fluoride concentrations (multi-standard addition mode) [17], sulphate in the presence of phosphate [18] and ammonium with a gas-sensing electrode [19].

*In vivo* determinations have been potentiated with the development of ion-sensitive field-effect transistors (ISFETs) [20], which allow a reduction of the size of potentiometric detectors to the point of facilitating their incorporation into hypodermic needles or probes. The improved signal-to-noise ratio yielded by this type of sensor is a result of the transistor output being located in the measuring zone as part of the electrode, in contrast to ISEs, in which the transistor output is located in the voltmeter, thus often picking up noise through the connection wires. Nevertheless, the success of ISFETs with *in vivo* measurements relies on the response to questions such as the influence of the flow (rate and angle of impingement) and proteins (membrane adsorption, viscosity, etc.) on the signal, the frequency of electrode recalibration and so on. All these questions have been brilliantly solved by Ramsing and Ruzicka [21] by using an automatic unsegmented-flow (FIA) system (Fig. 11.5). In the filling position (vertical position of the injection valve), the carrier, electrolyte or buffer circulates continuously through the by-pass where the reference electrode is located and thus continuously exposed to the same solution. In the injection position (horizontal position of the valve), the carrier passes through the valve inlet and sweeps the sample to the detector, which is thus exposed to a continuous flow simulating *in vivo* conditions, in which the ISFET is located in a continuous blood stream. This system allows investigations of the different variables affecting this type of *in vivo* measurement. Hence, it allows the selection of the electrode angle minimizing problems related to the flow-rate and, especially, it avoids the problems arising from the adsorption of red cells on the sensing membrane —one of the chief shortcomings of membrane electrodes, ISFETs and ISEs in general— by using a buffer or electrolyte as the carrier and connecting the injection port through to the blood stream, whence blood can circulate continuously through the injection system and be sampled at regular intervals (e.g. 1 min) by switching of the valve. In this manner, the detector membrane is exposed to the blood sample (5-30 μL) for only 10 s and to the buffer solution for 50 s prior to the following injection. The washing effect of the carrier solution frees the membrane from red cells, which are therefore prevented from gathering on its surface. Another advantage of this assembly is the easy recalibra-

tion of the electrode, accomplished by connecting a line carrying the calibra-
tion solution to the injection port. This ensures the calibration of the ISFET
at short intervals by alternate injections of blood samples and calibration
solution, and results in high precision. As claimed by its proponents, the
system allows the simultaneous determination of various species (pH, K, Na,
Cl, Ca, etc.) by casting different ion-selective membranes on the different
gates of the ISFET and using as little as 10 μL of sample per analysis —this
minimum sample consumption makes it unnecessary to return blood to the pa-
tient, thereby avoiding the need for sterilization of the detector.
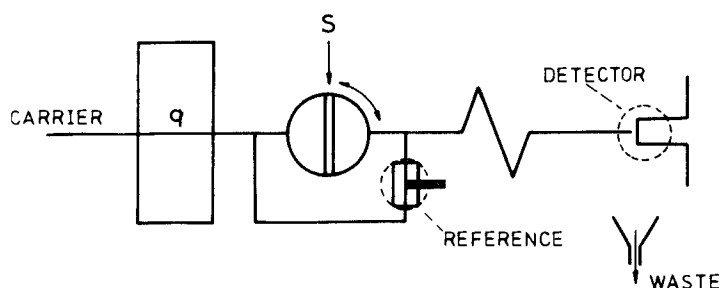


**Fig. 11.5** FIA configuration for *in vivo* measurements with ISFETs. The
peristaltic pump sets a carrier flow at a rate q. In the filling
position, the sample (S) circulates through the valve loop while the
carrier passes through the by-pass where the reference electrode is
placed. After switching the valve, a sample volume of 10 μL is driven
to the detector and then to waste with a residence time of 5–10 s.
(Reproduced from [21] with permission of Elsevier).

## 11.3 AUTOMATION IN VOLTAMMETRY

While potentiometry is possibly the most readily automatable electroana-
lytical technique, voltammetry is the one requiring most strongly the con-
course of automation. In fact, voltammetric measurements are generally complex
functions demanding greater efforts to obtain the desired analytical informa-
tion. This has moved workers for several decades now to investigate possible
solutions to the problems posed by the intrinsic characteristics of this elec-
troanalytical technique, as regards both sensors (working electrodes) signal
measurement and data processing. The advances in each of these fields have
materialized in the instruments launched recently, known as the 'second

generation of computer or microprocessor-controlled polarographs', of which the PAR 384B from Princeton Applied Research, the 646 VA Processor from Metrohm and the BAS 100 from Bioanalytical Systems are good examples. This section describes the evolution of research in each of the above-mentioned fields and comments on some commercial and non-commercial instruments of interest.

### 11.3.1 Working electrodes

Voltammetric working electrodes are generally characterized by their small surface areas, which result in improved polarization and minimum analyte depletion by electrolysis. The electrodes reported since the brilliant discovery of Heyrovsky can be classified into two groups: mercury or polarographic electrodes and solid electrodes. Both have experienced substantial improvements with time aimed at reducing or solving the shortcomings involved in their use.

Two of such improvements in the design of liquid electrodes have solved two major problems associated with these sensors, namely the control of the drop time and the rapid growth of the drop.

Obtaining a controlled drop time requires using a straightforward mechanism consisting of an electromagnetically actuated hammer hitting the electrode at preset intervals during the drop growth. The knowledge of the drop lifetime allows one to carry one current measurements over the interval best suited to the study in question —generally at the end of the drop lifetime, where surface changes are less marked and hence the contribution of the charging current is less significant. This design originally posed problems arising from the blow to the capillary in working at high sensitivities; the resulting increased capillarity effect caused undesirable convection phenomena in the liquid held in the cell, which in turn resulted in irreproducibility of the measurements. Moreover, there was some uncertainty in the drop time owing to the delay betwen the signal acting on the relay and its action. These problems have been overcome by current commercially available instruments such as the polarographic analysers 174A from PAR and E505 from Metrohm, equipped with electrodes in which the drop is released with the aid of a mechanized crescent-shaped clamping slider securing the electrode. At the preset time, the clamp securing the capillary performs a quick, short movement to separate the mercury drop from the end of the capillary in a highly precise manner. Another way of controlling the mercury flow involves the use of a solenoid valve or a peristaltic pump [22]. The use of electromagnetic devices has been avoided by detecting the drop fall through gravity and the surface tension rather than controlling the drop time. These mechanisms respond to the following principles:

(a) Overlap of a high-frequency a.c. signal and detection of the abrupt impedance change upon the drop fall [23]. The overlapped potential should be selected so as to avoid changes in the glass capillarity resulting from the effect of the thrust, but to ensure that it effectively separates each drop.

(b) Monitoring the abrupt intensity change at the end of the drop lifetime [24].

(c) Opto–electronic detection [25].

(d) Use of piezoelectric transducers [26]. A piezoelectric crystal of barium titanate built into the capillary holder produces a mechanical shock upon reception of a voltage pulse of about 200 V supplied by a computer–controlled timing system [27].
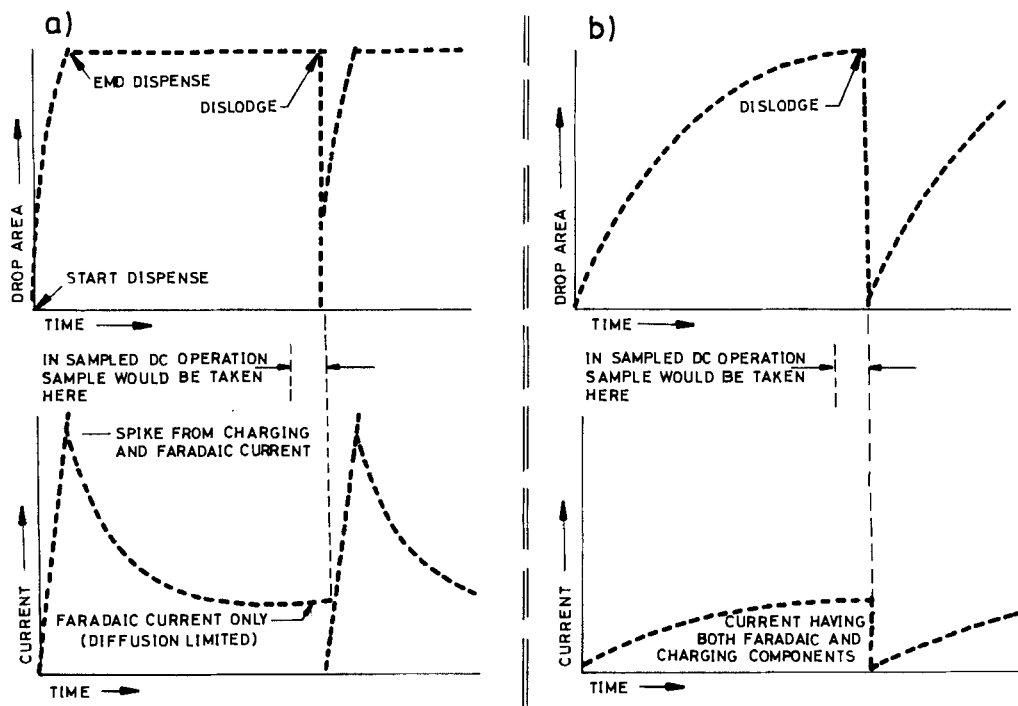


**Fig. 11.6** Drop area and current profiles of the static mercury drop electrode (a) and a conventional mercury drop electrode (b). (Courtesy of Princeton Applied Research).

Halting the rapid growth of the drop while its area is kept constant offers a number of advantages. As shown in Fig. 11.6, measuring the current immediately before the drop is released avoids undesirable currents —only the Faradaic current contributes to the measured intensity. This *modus operandi* results in an increased drop size —four times larger than those achieved by the
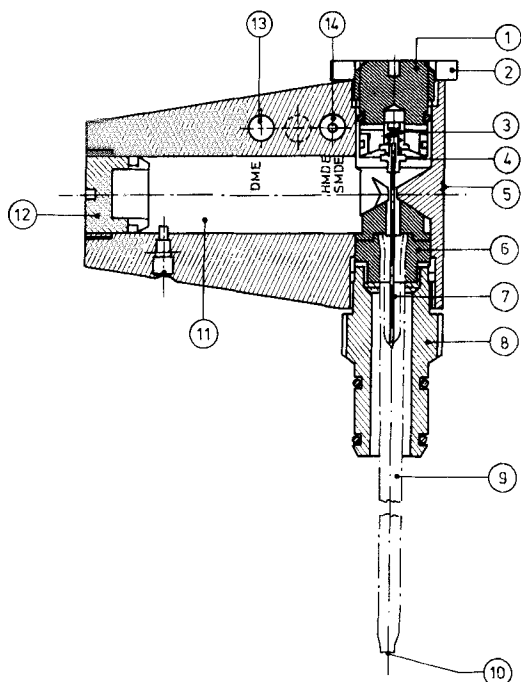
**Fig. 11.7** Scheme of a multiple mercury electrode. (1) Elloted screw; (2) screwed locking ring; (3) spring; (4) PTFE membrane; (5) flat front face; (6) silicone-rubber seal; (7) sealing needle; (8) retaining nut; (9) glass capillary; (10) tip of capillary; (11) mercury reservoir; (12) screw cover; (13) connection for N₂ supply when operating the electrode as a DME; (14) connection for N₂ supply when using the electrode as an SMDE or HMDE. (Courtesy of Metrohm).

procedures described above— and In greater baseline smoothness, which In turn gives higher sensitivity. In addition, the assembly allows the use of three variants of liquid electrodes: the dropping mercury electrode (DME), the hanging mercury drop electrode (HMDE) and the static mercury drop electrode (SMDE). A schematic diagram of one of these electrodes (a multiple mercury electrode, MME) Is shown In Fig. 11.7. The mercury drop Is pneumatically controlled by use of a nitrogen current to favour Its rapid growth and subsequent evacuation to preserve It from alterations. This type of electrode, marketed by firms such as PAR and Metrohm, had various precursors [28–30]. One such precursor was designed by Byers and Perone [31] (Fig. 11.8). It consisted of a sharpened stainless steel wire pulled In and out of a wide-bore capillary by a 6-V solenoid to valve the mercury flow. The capillary had an Internal diameter of 0.006 mm and was blown out at the end so that the wire was guided to the

orifice. The bottom 1.5 mm of the wire was sharpened to a fine point which extended 1 mm into the capillary beyond the point of contact. The electrical resistance measured between the mercury reservoir and the hanging mercury drop electrode was consistently about 5 ohm. The wire was sealed into a glass plunger with epoxy resin. A 6-V continuous duty solenoid lifted the plunger a distance of 2 mm and was activated by a computer-controlled relay from 0.1 to 0.6 s to form various drop sizes. The drop mass varied linearly with the solenoid activation time from 1 to 16 mg (standard deviation +0.1 mg). After the required electrochemical measurements had been made, a solenoid drop knocker struck the electrode to dislodge the drop.
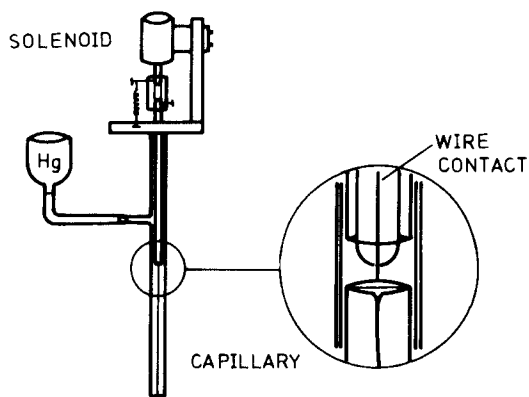


**Fig. 11.8** Scheme of home-made automated hanging mercury drop electrode. (Reproduced from [31] with permission of the American Chemical Society).

The chief problem posed by solid voltammetric electrodes is the lack of reproducibility arising from the sensor losses by blocking, poisoning or fouling. This problem is completely overcome by automation. The conditioning of the electrode surface, its coverage with an electroactive film and its clean-up can be carried out in a more or less automated fashion.

Poisoning of the electrode surface can be avoided or at least drastically reduced by applying polarization for very short times [32]. Mechanical devices [32,34] or even a proper cleaning brush can be introduced into the measuring cell to clean its internal surface with a rotating motion. Polarization by application of a potential with the opposite sign to that used in the analysis has been used, especially in cyclic voltammetry, to effect the electrode cleaning [35,36]. Such is the case with the Pt electrodes fouled during the oxida-

tion of phenol, which are freed from the adhering polymer membrane by anodic polarization [36]. Another procedure suggested for the automatic cleaning of Pt electrodes involves the periodic heating of the electrode in a gas phase [37,38].

### 11.3.2 Automation in data acquisition, processing and delivery

The unit relating the electrode potential to the time program is a key-piece in polarographic measurements. The earliest potential divider circuits using DMEs were manually operated and were soon superseded by synchron- or step–motor–driven potentiometers. Then came various program generators applying solid–state electronics which replaced mechanical potentiometers on account of the possibility of studying voltammetric phenomena through the different potential–time functions (sinusoidal and square–shaped waves of different frequencies, etc.) provided. It should be emphasized that fast polarization techniques owe their development to electronic program generators.

Voltammetric signals are the result of the current generated on application to the electrode of a suitable time–potential program. The generated currents can be measured with a variety of devices yielding more or less processed values.

The massive incorporation of microprocessors into analytical instrumentation may give the false impression than these devices have unlimited capacity. In fact, there are forms of polarographic instrumentation based on minicomputers and similar digital electronics that are capable of performing far more complex operations than microprocessors and that have been in use for many years. Microprocessors can only perform the same tasks in an electronically different manner at a price the manufacturer may consider economically viable for commercial production of the instrumentation. In other words, microprocessors have roused much interest only because they offer the same potential as microcomputers but at lower cost. However, their scope of application is not unlimited. The realization of a given task involves programming the microprocessor via its digital environment by using mathematical and logical operations written in assembler language or machine code. This type of low–level software is laborious to develop and rather costly. On the other hand, microcomputers are endowed with an electronic architecture that allows them to run sophisticated software under the control of an 'executive' program. This allows the programmer to command the computer to perform complex mathematical operations by means of straightforward line instructions in high–level languages such as FORTRAN, BASIC and ALGOL. No knowledge of machine code or assembler language is therefore required unless great speed and flexibility are the chief needs.

The above reasons have led workers to opt for mini- or microcomputers to perform tasks requiring the development of software and have resulted in the fact that most discussions on the use of computers and data treatment in voltammetry have arisen from results obtained from computer- rather than microprocessor-controlled systems.

An interesting contribution to data processing is the work by Bond [39], in which the performances of different variants of the voltammetric technique (semi-intergral, semi-differential, direct current linear sweep, direct current derivative linear sweep and pulse and related techniques) were compared with the aid of computerized instrumentation.

Semi-integral or deconvolution and semi-differential or convolution voltammetry are two closely related techniques insofar as the latter derives from the former [40–49]. Linear sweep voltammetry is an intermediate variant lying between the two but which is easier to implement because the current is the quantity directly measured and displayed —in the two above-mentioned techniques the current is first measured and then differentiated or semi-integrated via an analogue or digital method. Derivative (first or second) linear sweep voltammetry [50–54], in its differential  and integral variants, is another electroanalytical technique closely related to the previous ones and derived from the current-voltage curve in linear sweep voltammetry over which it is intended to improve [55]. Linear sweep techniques using linear potential-time voltage ramps feature lower Faradaic current/charging current ratios than their counterparts using staircase or pulsed potentials [56–60] to generate d.c. potentials and are therefore less sensitive. Although, in principle, semi-derivative, semi-integral and derivative variants of the staircase or pulse techniques would be theoretically feasible, the change from linear to pulse d.c. ramps results in altered time scales and requires new considerations to be applied.

Just like unique and technique-dependent electronic developments were required in the analogue work, few of these techniques and/or concepts are theoretically independent. In fact, for a given d.c. waveform and electrode process, all the responses of the different modes can be generated from the same experimental data and, in principle, the difference would only lie in the data processing procedure. This hypothesis is difficult to test experimentally with analogue circuits. However, computerized instrumentation and digital electronics make data collection and storage rather an easy task and therefore facilitate the sequential generation of semi-derivative and semi-integral curves and their first or subsequent derivatives by mathematical manipulation of unprocessed data.

The accuracy of the above assertions was checked by Bond [39] with a
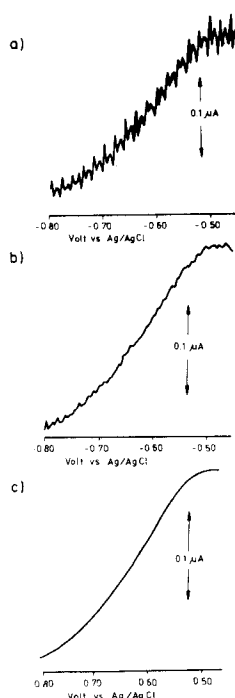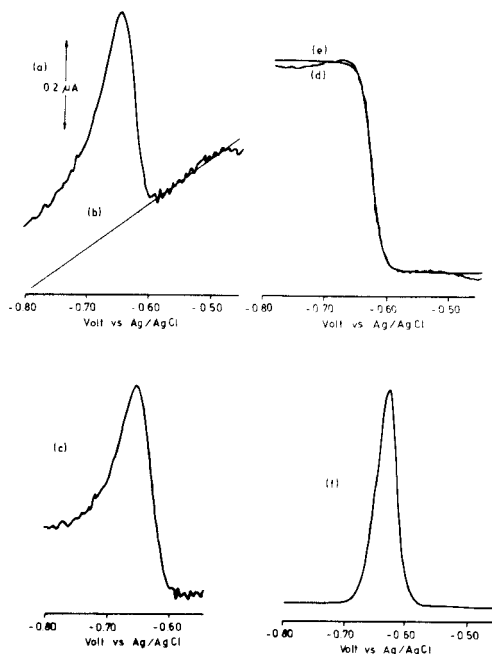
**Fig. 11.9** Background current in linear sweep voltammetry in 1M NaCl at a DME. (a) Raw data: 1 scan from 1 mercury drop. (b) Average of 25 scans from 25 mercury drops. (c) Data average by RC damping. (Reproduced from [39] with permission of the American Chemical Society).

computerized electroanalytical assembly consisting of a PAR 174 Polarographic Analyzer interfaced to a PDP-11 minicomputer. He used a microprocessor-controlled function generator [56] to generate staircase waveforms. The program governing the operation of the polarographic analyser as regards data acquisition and evaluation, background current correction and data display was written in BASIC by using the set of subroutines available from the PAL II assembly language [57,58]. The assumption that the above-mentioned voltammetric techniques derive directly from the current-potential curve obtained in linear sweep voltammetry is confirmed by the fact that the same background noise levels of the different signals are obtained by mathematical manipulation of the supposedly precursor signals. In constructing analogue circuits for the development of each technique, different RC network characteristics or different charging current correction approaches can lead to apparent detection limits resulting from the instrumental design rather than from the underlying method. Figure 11.9 shows the i-E background or charging current obtained for

a 1M NaCl solution in the absence of an electroactive species. Figure 11.9a corresponds to the single-pass data collected with no deliberately applied filtering other than that inherent in playing back data at slow speeds on an X-Y recorder. Figures 11.9b and c show the average results of 25 duplicate experiments and of the RC filtered data, respectively. The original, unaveraged data contain considerable electronic noise which can be supressed by RC damping or minimized by ensemble averaging. The background current can be stored in memory and subsequently subtracted from the data to improve d.c. experiments. Alternatively, corrections can be made by computing the linear least-squares or quadratic least-squares fit of data to the potential removed from the Faradaic current (Fig. 11.10). Such computations enable predictions to be made concerning the background current potential associated with the Faradaic current [57,58]. Occasionally, background level corrections carried out in this manner are to be preferred to those made by storing values in memory and subsequently using them directly for subtraction [58].



**Fig. 11.10** Linear sweep semi-integral and semi-differential curves of $10^{-5}M$ Cd(II) in 1M NaCl at a DME. Average of 25 scans. (a) Linear sweep curve. (b) Linear extrapolation of background current. (c) Background-corrected linear sweep curve. (d) Semi-integral curve. (e) Least-squares fitted semi-integral curve. (f) Semi-differential curve. (Reproduced from [39] with permission of the American Chemical Society).

Studies made with this instrumentation on other voltammetric techniques such as anodic stripping voltammetry allow one to conclude that the optimization of initial d.c. linear sweep or stripping data leads to optimum performance in the semi-integral, semi-differential and derivative approaches and that, under instrumentally equivalent conditions where d.c. experiments have been optimized with respect to electronic noise and background correction, detection limits are not markedly different within the sub-set of related approaches. Obviously, the resolution and ease of use of a method providing a peak-type readout (semi-differential) are superior to those with sigmoidally shaped read- outs (semi-integral).

Another major advantage of the automation of data acquisition and treatment is the evaluation of overlapping peaks or curves, for which Bond and Grabaric [59] have proposed a method based on the subtraction of polarograms related by two electrodes. Thus electrode potential-current intensity data corresponding to a sample containing two species A and B can be stored in the polarograph memory. After preparing an identical sample containing species A only, its polarogram is subtracted from that obtained for the mixture to obtain that corresponding to species B. Other more complex methods for the resolution of overlapping voltammetric curves based on the use of partial simulated voltammograms and real curves have been reported by Perone and co-workers [60,61]. They used the numerical deconvolution technique for joint analysis for major and minor components (in ratios of up to 1000:1 and with peak potential differences of up to 150 mV) and for components with very close peak potentials differing by only 35-40 mV. The voltammograms were described by equations in which the values of the constants depended on the particular component. By using the values stored in the computer, standard polarograms were generated and their combination was fitted to the polarogram of the sample. Strongly overlapped curves or peaks (differences of 4-12 mV) were tackled with pattern recognition techniques, with which a classification with a reliability of greater than 90% was established by using real [62,63] or real and simulated data [64].

Some specific applications of voltammetry require the use of more sophisticated programs and computers. Such is the case with the instrumentation used for on-line fast Fourier transform (FFT) data processing. The use of a digital FFT as an on-line data processing strategy has met with wide acceptance in the fields of nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy. However, on-line digital FFTs have rarely been applied in electroanalytical chemistry, despite their proven advantages.

### 11.3.3 Instrumentation

The design of a microprocessor- or computer-controlled polarograph corre-

sponds basically to the scheme in Fig. 11.11. The computer (microprocessor) is initially used to apply a digital potential —previously converted to analogue form— to the potentiostat. The readouts from the i-E curve, carried out on an X-Y recorder or an oscilloscope in the case of analogue instruments, are converted from the analogue form provided by the potentiostat to a digital form intelligible to the computer by means of an A/D converter. In contrast with the subsequent treatment required by curves obtained with an analogue system (*viz.* obtainment of $E_{1/2}$ and $i_D$, correction for the $R_i$ drop, etc.), the data acquired by the computer are the starting point for the automatic system: the software allows for any intermediate calculation by use of commands issued by means of the keyboard or a teletypewriter.
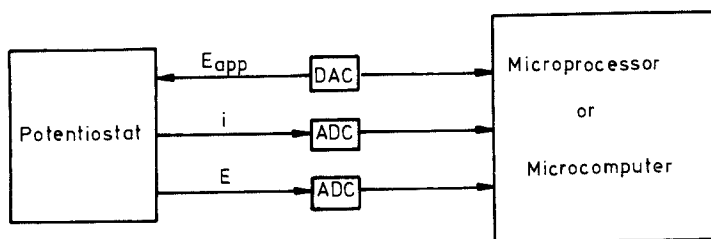


**Fig. 11.11**  Scheme of an automated polarographic analyser.

This type of instrumentation is varied and features a variable number and ratio of analogue-to-digital components. Below are described some representative examples of automatic polarographic analysers.

Figure 11.12 shows the scheme of the set-up used for current-sampled d.c. polarography designed and constructed by Bos [65]. It consists of a PDP 11/20 computer, an ASR 33 teletypewriter, a Dectope TU 56 unit, AD01 analogue-to-digital and AA11D digital-to-analogue converters and a DTL 1  drop life timer. The instrument operates according to two programs performing two sequential tasks: control and acquisition of the signal-response and evaluation of the results obtained. The program controls the maintenance of the potential difference between the working and reference electrodes at a preset value with the aid of the auxiliary electrode, linear changes with time of the potential of

the working electrode, measurement and storage of the current-time relation-
ship, synchronization of the measurement of the cell current with the lifetime
of the mercury drop and release of the drop at preset intervals. The second
module of the program allows the obtainment of i-E values for all the points
on the rising portion of each polarographic curve and for the limiting
current, in addition to the half-wave potential and the slope of the E *vs.*
log [(i$_D$-i)/i] plot. It also permits correction of the baseline by extrapola-
tion to a least-squares line consisting of 20 points obtained from 40 points
preceding the rise of the wave. The results obtained with this instrument
compare favourably with those provided by conventional analogue instruments.
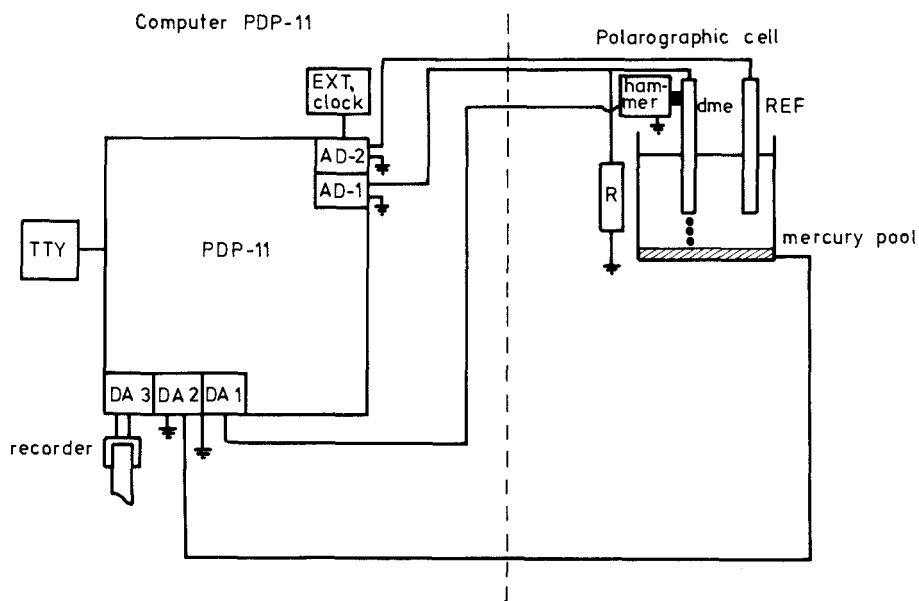


**Fig. 11.12** Scheme of the computer-controlled polarograph designed by
Bos. (Reproduced from [65] with permission of Elsevier).

The one-drop square wave analyser designed by Osteryoung and co-workers
[66,67] filled the gap the specific commercial instrumentation left in this
voltammetric mode. The instrument, depicted in Fig. 11.13, has the following
features: a symmetrical square wave with a period of 1/60 s and a fixed-wave
peak-to-peak amplitude of 50 mV, providing a reasonable compromise between
resolution and sensitivity. The remainder of the experimental parameters (e.g.
the step height, initial potential and delay time) are selected by the oper-
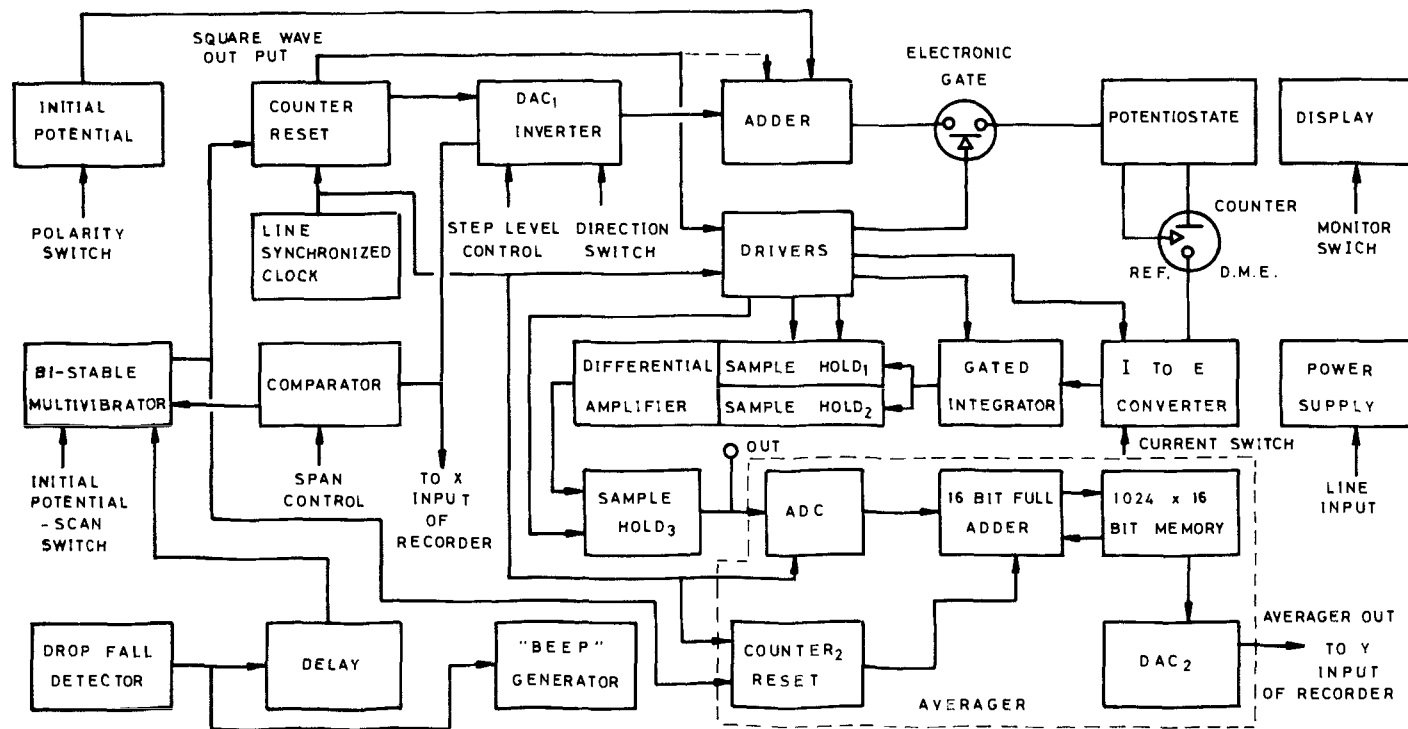
**Fig. 11.13** Block diagram of a one-drop square wave analyser. (Reproduced from [67] with permission of the American Chemical Society).

ator via the controls on the instrument front panel. Up to 30 scans are possible. Its application to the analysis of flow systems [68] and the oxidation of mercury in the presence of chelons [69], and its joint use with rapid deaeration devices [70–72], confirmed its expected sensitivity and rapidity and resulted in noise levels lower than those typically present in more flexible multi-purpose systems.

The modular interface constructed by Baldwin and Price [73] allows the acquisition and processing of data by use of a TRS-80 microcomputer and any analogue electrochemical analyser. The system requires no knowledge of assembly language programming as it uses BASIC throughout, even for data acquisition. Twelve-bit acquisition rates as high as 60 Hz are affordable, thereby making the system suitable for most routine electroanalytical applications.

A programmable waveform generator capable of producing complex voltage-time programs based on a PDP-11/34 minicomputer with associated digital hardware has also been reported. The desired waveform is obtained by clock-controlled output of a symmetrical triangular voltage sweep. The software can be readily adapted to any computer system. Ramp sections of 1 mV/s to 50 V/s and potential pulses of 50 μs to 500 s can be easily obtained.
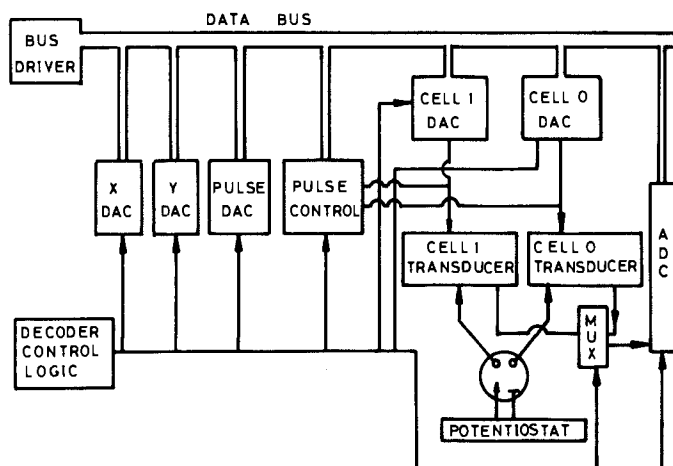


**Fig. 11.14** Computer-controlled twin working electrode potentiostat. (Reproduced from [75] with permission of Elsevier).

The twin-electrode voltammetric mode has also been automated by means of a home-made microprocessor-controlled potentiostat [75]. The computerized control of the system is required for the application of complex waveforms and timing sequences to the twin working electrodes in order to eliminate interferences due to the formation of intermetallic species —the chief application of this electrode set-up. The features of this instrumental assembly include the use of a single potentiostat with four electrodes (reference and auxiliary plus two working electrodes), allowing the independent control of each working electrode, and an interface to a 6502 microprocessor permitting the application of an unusual voltammetric mode with any number of potential waveforms and current-sampling methods, and 60-Hz line noise elimination. A block diagram of the interface linking the microcomputer with the electrode cell is shown in Fig. 11.14.

Complex studies such as those carried by Danielsson *et al.* [76] on the applicability of the superimposition principle in differential pulse polarography required the joint use of two computers. The voltammetric set-up used by these workers was based on a minicomputer system (Intel SYS–80/10A computer
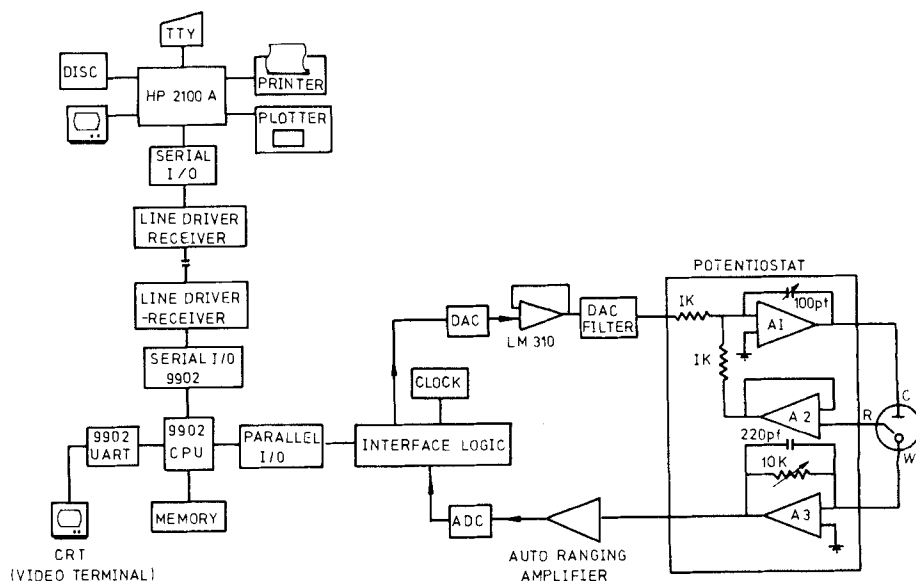


**Fig. 11.15** Block diagram of mini-/microcomputer-controlled voltammetric system. A1, A2 and A3 denote analogue devices 120A, Burr Brown 3013/15 and Burr Brown 3064/15, respectively. (Reproduced from [79] with permission of Elsevier).

Interfaced to a potentiostat and an I/E converter via a 16-bit D/A and a 12-bit A/D converter) to handle real-time operations and a personal computer (Luxor Scandia Metrid ABC80) to set up experiments and for calculation of results [77]. The measurement and control program used was an extension of a previously written program for pulse voltammetry [78]. Two computers were also used for the semi-hierachical computer network for data acquisition and control in staircase voltammetry constructed by Li *et al.* [79] according to the scheme in Fig. 11.15. The system was connected to a Hewlett Packard 2100A minicomputer via a Texas Instruments 9900 microcomputer and a bidirectional

Start

DAC and Clock Set Up Routine
- Wait for initialization values for DAC and clock
- Initialize DAC and clock

Run Data Acquisition Routine
- Wait for initial instruction and data pointers
- Activate the clock

Instruction Interpreter Routine
- Decode instruction
- Find address of service routine
- Decode argument

- Execute one of the service routines

Is Command Field = 0? — No

Yes

Exit

**Fig. 11.16** Flow-chart of the execution sequence of the real-time interpreter of the system in Fig. 11.15. (Reproduced from [79] with permission of Elsevier).

serial transmission link. The experimental parameters were down-loaded from the host to the satellite, which supervised the experiments at a remote location. The data collected were transmitted back to the host at 2400 Baud for reduction and plotting. Through this division of labour, each system was used to its best advantage. Although the software was developed specifically for staircase voltammetry, the system hardware was of general-purpose design and therefore suitable for other types of pulse experiments. The real-time executive sequence of the flow-chart is shown in Fig. 11.16. Conversely, the instrument designed by Danielsson *et al.* [76] required no complex multi-task system or remote host computer [77].

*In vivo* voltammetric measurements have also been automated with the aid of custom designs. This type of measurement demands some features from the instrumentation to be used, namely: (a) the possibility of using several working electrodes simultaneously; (b) accuracy in the current measurements to within a few nanoamperes; (c) complete automation for long-term experiments; (d) rapid gain adjustment for different response levels. The recent advances in large-scale integrated (LSI) circuits and the accessibility of versatile commercial microprocessors make the above demands affordable. The system designed and evaluated by Adams *et al.* [80] for *in vivo* studies, shown schematically in Fig. 11.17, uses a potentiostat controlled by a microprocessor via a user-designed interface for electrochemical studies on the brains of non-anaesthetized animals. The potentiostat [81] features four independently operated current followers which allow the currents at four different working electrodes implanted in different parts of the brain to be controlled simultaneously. The voltage (0 to +1.0V) is applied via a D/A converter, while the output current has a fixed gain of 10 mV/nA. These conditions were optimized for the oxidation of monoamine neurotransmitters and metabolites in graphite microelectrodes 100-200 μm in diameter. Smaller electrodes (8 μm) require modification of the current follower to accommodate the decreased current. A rapid solid-state delay is inserted between each working electrode and its corresponding current follower. All the working electrodes are kept at open circuit until the relays are commanded by the microprocessor to close them momentarily. Figure 11.17b shows the major components of the system and their functions in relation to the microprocessor and potentiostat. The system monitor, furnished by Intel, is stored in an 8355 ROM. With appropriate strapping connections, the monitor's utility routines service the keyboard, the LED display and the line printer. A 2716 EPROM contains the user's program, in 8085 machine language, to perform the electroanalytical experiments. The programs are written in 8085 assembly language. The address field and data field of the on-board LED are used for displaying the time elapsed and the number of runs performed.

a)



b)



**Fig. 11.17** (a) Scheme of the instrumentation used for *in vivo* electrochemical experiments. (b) Potentiostatic circuit and microprocessor interface (the electronic components are shown for one of the electrodes only): SDK-85, Intel microcomputer system; 7520, D/A converter; A₁ and A₅, 741 analogue devices; A₂ and A₄, Burr Brown 3510; A₃, Burr Brown 3527; VFC-32K, Burr Brown V/F converter; 2716, Intel EPROM; 8253-5, Intel counter; DAC-08, PMI D/A converter; R = 1 mΩ, 1%. (Reproduced from [81] with permission of the American Chemical Society).

Each run, consisting of four simultaneous chronoamperometric measure-
ments, is performed automatically at the programmed time interval. This new
approach has been used to show a highly significant differential response of a
neuroleptic drug by simultaneous recordings in two different areas of rat
brain [83].

Possibly the most promising aspect of *in vivo* voltammetric measurements is
voltammetry in flowing solutions, a thorough revision of which has been re-
ported by Johnson *et al.* [84].

'Second-generation' commercially available analysers are all highly auto-
mated instruments accommodating a microprocessor for controlling the analogue
potentiostat in carrying out the different voltammetric techniques, namely the
pulse, square, triangular and sinusoidal wave and differential and stripping
modes. Determining the height and position of the peak, subtracting the back-
ground and re-scaling the I-E curve are all carried out under the micropro-
cessor's control. The 646 VA Processor with the 647 VA Stand, which accom-
modates both the MME and the RDE, are a representative example of this type of
analyser. The instrument is controlled from a 16-bit microprocessor system
consisting of CPU, ROM and RAM, in addition to various interfacial circuits
linked together via bus lines. The dialogue with the instrument (man-machine
communication) takes place through the monitor screen, the alphanumeric key-
board and the printer/ plotter. The user can store numerous methods complete
with all the necessary parameters, adjustments and remarks in the large non-
volatile memory. A total of two 647 VA stands can be connected to one 646 VA
processor. In this way it is possible to alternate between analyses and to
prepare the next sample manually. On each 647 VA stand the MME electrode can
be operated in different electrode modes. Its various auxiliary functions
(e.g. purging, opening of the Hg valve, stirring) are activated through the
printed circuit boards. Input signals such as 'overload stirrer' or 'overload
iR' are passed to the bus lines through the same interface. Up to four motor-
ized piston burettes can be connected to each of the two Dosimat interfaces,
giving a total of eight units. The data for volume and dosing are predeter-
mined and the operating and control elements of the Dosimat are interrogated.
The weighing data of an electronic balance can be fed into the microprocessor
of the 646 VA processor through the balance interface. Data can be exchanged
with an external communication unit via the RS-232 or the IEEE 488/IEC 625
interfaces. They are intended to connect and control a future VA Sample
Charger through an additional interface. This type of analyser is also
represented by the PAR (Princeton Applied Research) Models 384-1, 384-3 and
384-4. These are fully automated instruments operating under external computer
control, with digital data output and the possibility of incorporating the

SMDE Model 303 and implementing up to six different techniques. Even more powerful is the BAS-100 Electrochemical Analyzer from Bioanalytical Systems, capable of implementing up to 29 different techniques under the control of its built-in microprocessor. It can be linked to a personal computer fitted with disc drive. BAS also market software for use with their system and an IBM PC or Apple IIe computer.

## 11.4 AUTOMATION IN STRIPPING ANALYSIS

Voltammetric, potentiometric and coulometric stripping analysis are dealt with here in order of increasing importance, which is also the chronological and developmental order. Voltammetry is the oldest of these techniques and the only of the three that has given rise to commercially available automatic instruments. In fact, virtually all the 'second-generation' automatic voltammetric analysers feature some variant of the stripping technique in their menus, a technique which is endowed with the inherent advantages of these instruments (background correction, measurement averaging, etc.).

Kryger and co-workers contributed significantly to the developments in this field in the 1970s [85-87]. The most interesting of their contributions was the automation of multi-scanning, background-subtraction stripping methods. According to an automatic measurement program, a de-aerated and stirred $Hg(NO_3)_2$ solution was used to electrically deposit an Hg film on the surface of a vitreous carbon electrode which was introduced into the measuring cell. This contained Cd and Pb, for instance, and was applied a potential of -1.3 V for 2 min, with which the electrode surface was enriched with the metals in the solution, which formed amalgams. According to the usual stripping analysis procedure, the solution was then kept at rest and the working electrode was applied a scanning potential between -0.9 and 0.2-0.3 V for 0.2-0.5 s. Then came the reduction at a potential of -1.25 V and a new scan. In this way, the stripped ion remaining in the diffusion stage were repeatedly reduced. The scanning and reduction cycles were repeated several times (20-40), the computer recording the current intensities measured along the scan and storing them in memory. The next step involved rotating the working electrode for a few seconds while keeping the potential at -0.01 V. The scanning cycle was then repeated as many times as before, the computer gathering the background current intensities. The signal-to-noise ratio obtained after the background correction was good, allowing the determination of less than 1 ppb of Pb or Cd [85-87].

Brown and Kowalski [88] designed a minicomputer-controlled anodic stripping analyser consisting of the following elements: a home-made, computer-con-

trollable potentiostat, a general interface and a Digital Equipment PDP 11/05
GT 40 minicomputer equipped with 24k core memory and hardware arithmetic
capability. The technique employed used differential voltammetry at one
electrode and a rapid data-averaging algorithm. With these, a signal-to-noise
ratio better than that of the above-described multi-scanning method, an excel-
lent detection limit and sensitivity and an acceptable reproducibility of 5%
were achieved. The program controlled the deposition time and electrode rota-
tion, performed the scan by using a variable-point averager and then carried
out the background scan, again by using point averaging and Fourier domain dig-
ital filtering on request.
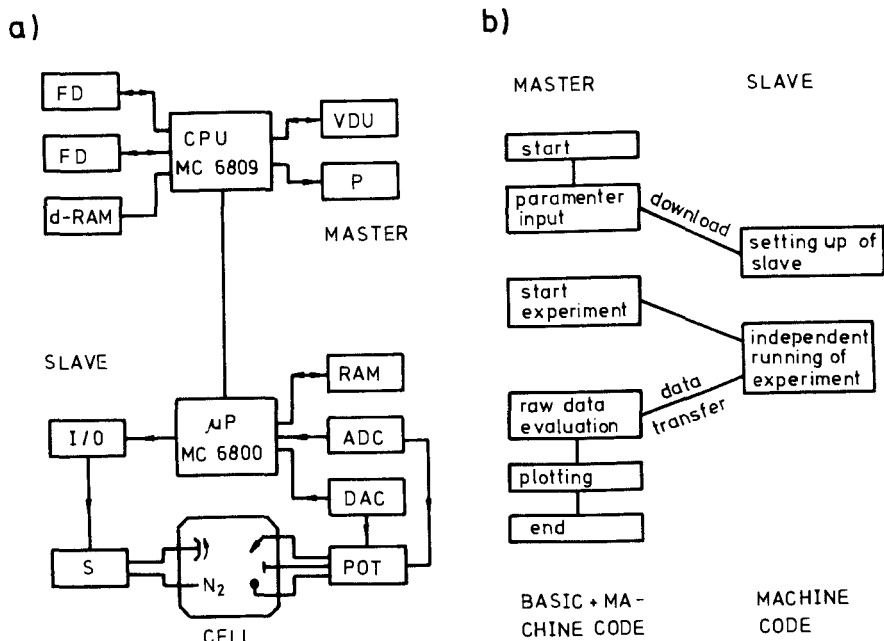


**Fig. 11.18**  (a) Block diagram of the distributer microprocessor system
and cell of the stripping set-up used by Bond *et al* [89]. Master
system: CPU, MC 6809 central processing unit; VDU, visual display
unit; P, printer; FD, floppy disc; d-RAM, dynamic RAM. Slave system:
uP, MC 6800 central processing unit; I/O input-output board; S,
control devices for nitrogen purge and stirrer; POT, potentiostat;
ADC, analogue-to-digital converter; DAC, digital-to-analogue con-
verter. (b) Flow chart describing operation of master and slave compu-
ter. (Reproduced from [89] with permission of Elsevier).

A home-made instrument of this type for use in chemically hazardous, ra-
diation or clean laboratories was recently reported by Bond *et al.* [89]. Its

block diagram and flow chart are depicted in Figs 11.18a and b, respectively. The instrumental set-up is divided into two chief sections: (a) the master and (b) the slave system. The slave system is based on the laboratory where the experiments are carried out, while the master resides in the remote computer room. The slave system consists essentially of a cell, a potentiostat and the slave, *viz.* a modified Mottorola MEK-6800-D2 kit microprocessor system. The 6800 system contains 16 kb of RAM and an input-output board containing a 12-bit DAC and ADC. In addition, some external control lines are available (e.g. for rotating the electrode and opening and closing the nitrogen valve). All programs are designed to be used with controllable mercury drop electrodes. This section is conceived for special cases of d.p.a.s.v. experiments; implementation of other techniques requires only software changes. The master system is located in an air-conditioned room. It consists of a Sphere 6809 microprocessor system running Flex 9.0 and an operating system. There is 256 kb of dynamic memory, of which approximately 193 kb are incorporated as a virtual disc. Two DT/8 floppy disc drives and a matrix printer are connected as peripherals. The master system is linked by RS-232 interfaces to each slave system. Machine code is used in the slave system and for communication to the master system. Both machine and high-level languages are used by the master. The flow chart for an experiment controlled by this distributer microprocessor system is depicted in Fig. 11.18b. The direct current component of the d.p.a. s.v. is collected just before applying the pulse and subsequently pulse currents are measured at 20-ms intervals during the pulse life. The experiment is started at the master system. Once initiated, the slave runs the entire program independently and the master system can then be used for any other task. Because the slave has its own memory, it can store the data indefinitely until transmission is requested by the master computer. The fact that the inexpensive microprocessor system can operate independently makes it possible to use several slave systems simultaneously. This would be impossible if the master system directly controlled both the experiment and data acquisition. One set of raw data is stored in a file on a floppy disk and a second set is generated and converted to a format suitable for plotting. These data are plotted on the printer or any other graphical unit.

Voltammetric stripping analysis has scarcely been applied to automatic *in vivo* measurements. The research by Wang [90] is an exception than opens interesting prospects in this field.

Potentiometric stripping analysis (PSA), although introduced by Bruckenstein and co-workers [91,92] in the early 1960s, was not used for analytical purposes until the middle 1970s by Jagner and co-workers [93,94], hence its comparatively scarce development —both in the theoretical and in the instru-

mental respects. The PSA technique, commonly applied to metal ions and, less often, to non-metals (cathodic potentiometric stripping), involves a precon-centration stage similar to that included in voltammetric stripping, after which the electric circuit is open and the reverse reaction of the deposition takes place spontaneously with the aid of atmospheric oxygen or a redox agent added to the sample. The recording obtained is compared with that typically provided by the voltammetric mode in Fig. 11.19.
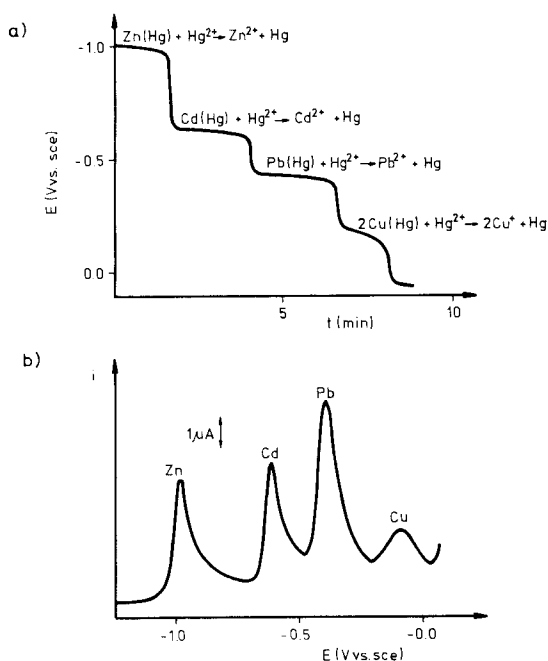


**Fig. 11.19** (a) Recording obtained in the potentiometric stripping of a mixture of metal ions. (b) Recording obtained by voltammetric strip-ping analysis of the same mixture.

The analogue instrumentation involved in the implementation of this tech-nique is fairly simple, although it poses problems derived from the difficulty of controlling the plating potential and time and the low resolution of time from the x–t recorder. These shortcomings can be brilliantly circumvented by using a microcomputer to control the analysis and other functions such as sam-ple change-over, reagent dispensation and result evaluation, thereby consider-ably reducing the analysis time. Anfält and Strandberg [95] demonstrated the advantages of the automation of PSA in designing and using the microcomputer-controlled system shown in Figs 13.20a and b, which depict the analogue sec-

tion of the system and its block diagram, respectively. Taking full advantage of the assets offered by the built-in computer requires re-programming, which can be carried out in a high-level language such as BASIC.

a)



b)
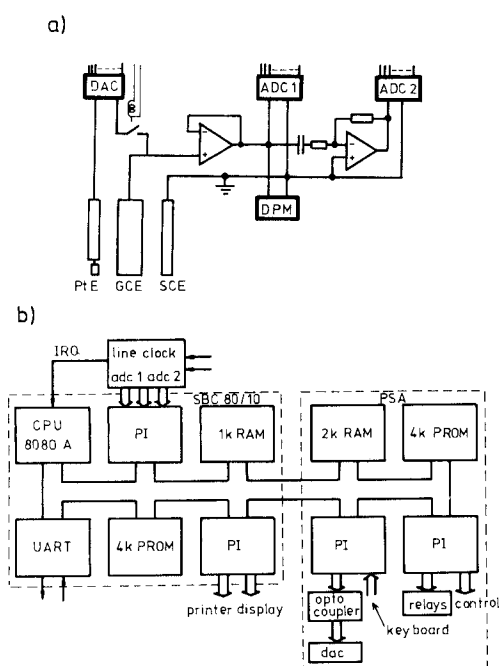


**Fig. 11.20** (a) Analogue section of an automated PSA instrument. The operational amplifiers are NS LF 336. The digital panel meter and the differentiating amplifier are optional. (b) Block diagram of the instrument. PI, parallel interface; IRQ, interrupt request. Only 4 kb worth of the PROM capacity is used. Bus transceivers are not shown. (Reproduced from [95] with permission of Elsevier).

The multi-scanning variant of PSA has also been implemented with the aid of a computerized data-acquisition system. The improvement in the analytical signal achieved depends closely on the recovery (re-reduction) of the metals re-oxidized after each cyclic scan. If the time available for the oxidized metal to separate from the electrode by diffusion into a quiescent solution is short, its recovery will consequently be high. Preconcentration periods of 30 min result in detection limits of 5 ng/L for Cd and Pb [97]. A computerized system implementing the conventional PSA mode was used by Jagner *et al.* [97] for the determination of these two metals in blood and serum with clear advantages over the voltammetric technique as regards selectivity, sensitivity and sampling frequency.

Coulostatic stripping analysis was developed as a response to a general problem affecting stripping techniques in which, because of the change of the potential with time, the current always flows through the solution with an ohmic drop resulting from the solution resistance. Part of the current charges the double layer, which results in errors in the analytical determination insofar as only the total current can be measured. The coulostatic technique completely eliminates the resistance of the solution not compensated for by the application of a charging pulse to the cell, which rapidly charges the capacitance of the double layer, $C_{dl}$. Once the double layer has been charged, there is no current flux through the solution or uncompensated solution resistance. The potential measured at the reference electrode coincides with the potential across the double layer. If the potential of the double layer resulting from the application of the charging pulse is sufficiently high, the Faradaic current will flow through $R_f$ and discharge $C_{dl}$. The resultant transient can be used to evaluate the Faradaic current. This can be done by fitting the curve to a theoretical expression in order to obtain the instant current or by measuring the total charge contained in the transient and computing an average current. The capacitance of the double layer can be calculated from the pulse charge, Q, and the potential change, $\Delta E$, resulting from the applied pulse, according to the equation

$$C_{dl} = Q/\Delta E$$
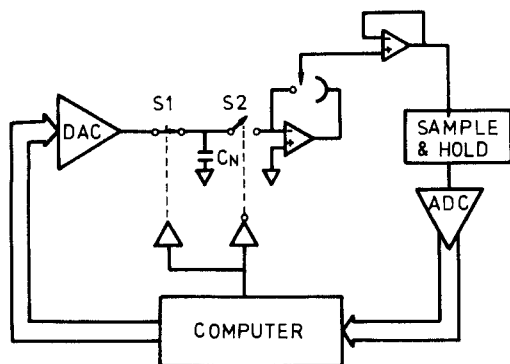


**Fig. 11.21** Block diagram of a computer-controlled coulostatic analyser. DACm, 12-bit D/A converter; S1 and S2, TL 191 analogue switches; ADC, 12-bit A/D converter. (Reproduced from [98] with permission of the American Chemical Society).

Figure 11.21 shows a schematic diagram of a computer-controlled coulo-

static system. An LSI-11 microcomputer controls the instrument and effects data acquisition and reduction. The instrument can implement different electrochemical procedures by changing the way in which the charging pulses are applied to the electrode. In addition, switching from mode to mode merely requires changing the software. In this way chronopotentiometric and linear sweep anodic stripping modes can be applied [98]. The instrument features a precision of 5% for 1 ppb Cd in the linear sweep anodic stripping mode with a thin-film mercury/wax-impregnated graphite electrode, which corresponds to a detection limit of 100 ppt. Even greater sensitivity is achieved by adding the data corresponding to multi-scanning stripping analyses.

## 11.5 AUTOMATION IN SPECTROELECTROCHEMISTRY

Spectroelectrochemistry [99] is a hybrid technique resulting from the association of electrochemistry with spectroscopy via the use of cells with optically transparent electrodes [100-103]. The potential of this technique lies in the possibility of identifying both the type and the amount of the species generated in an electrochemical step. The intrinsic characteristics of spectroelectrochemistry require the use of fast measuring systems —spectroscopic image detectors in most cases [104-107]— and the consequent acquisition of the large number of data provided by the detection system in a short time by means of an oscilloscope or, even better, of a computer also allowing the subsequent exhaustive treatment of the raw data.

Figure 11.22 shows the design of the rapid scan instrument for spectroelectrochemical measurements in the visible region reported by Wells [108] in 1973. It consists of a monochromator designed by Strojek *et al.* [109], a Digital Equipment PDP-8/I computer equipped with an AX-08 8-bit analogue-to-digital converter, digital and pulse input and output, clock, oscilloscope and an AA-01 12-bit converter. The potentiostat system is a Tacussel PIT 20-2A with a GSTP-2 waveform generator. The ramp generator for the spectrophotometer wavelength drive and the potentiostat pulse generator are each controlled by a trigger pulse from the computer. The species generation time, which is the interval between the two trigger pulses, is controlled by the computer through its internal real-time clock. Allowable values range from 100 ms to a few seconds, limited by a desire to keep the scan fast relative to the change in concentration of intermediate in the first case, and by convention of the solution in the latter. After the wavelength scan is initiated, the absorbance signal is collected by the A/D converter and stored in memory for possible averaging with latter runs. The D/A converter output, shown mixed with the absorbance signal prior to collection, provides dynamic baseline control. Data

obtained from the experiment are logged on the teletype and plotter. The electrochemical cells used are of the transmission type, in which the beam from the monochromator passes through an optically transparent Pt electrode. Nothing in the design of the system limits sampling by reflection or attenuated total reflection. The instrument is capable of recording spectra of species with half-lives of the order of a few milliseconds. It does so by using the unique capabilities of the computer to remove the baseline irregularities which limit sensitivity of the spectrophotometer alone, and by signal averaging. Moreover, the system is capable of managing the very complex *in situ* spectroelectrochemical experiment to obtain results that are virtually impossible to accomplish manually.
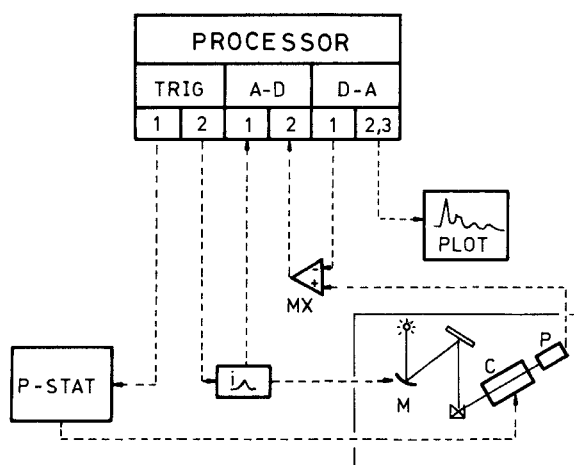


**Fig. 11.22** Scheme of microprocessor-controlled spectroelectrochemical system. (Reproduced from [108] with permission of the American Chemical Society).

A more recent instrument of this type was reported by Strojek *et al.* [110] and is depicted in Fig. 11.23. The spectrophotometer is controlled by a Nova-2 minicomputer equipped with two 8-bit D/A and A/D converters. The electrochemical cell is controlled by a PRT-100-1X Tacussel potentiostat. The measured curves are recorded by means of a Yokogawa x-y recorder or a 141B Hewlett-Packard storage oscilloscope. Data are also output by a line printer or on punched tape. The controlling program is introduced into the computer's memory by means of a fast paper-tape reader. The minicomputer is connected to the spectroelectrochemical system by four lines; two D/A converters are used to control the electrochemical process and one 2-input A/D converter serves to collect the results. The output OUT1 controls the potentiostat and the input IN1 is used to

record the spectrochemical data. The analogue output OUT2 controls the galvano-meter mirror of the spectrophotometer, and the input IN2 is used to record the spectral data. The spectrochemical process is controlled by a Spectrum program written in the computer's native code; it is added to the BASIC interpreter in the form of sub-programs. The complete control cycle consists of W steps in time T. At each step, the minicomputer sends a voltage controlling the potentio-stat to the output OUT2, and receives the current intensity of the voltage of the working electrode via input IN1. The program can measure a 256-point spec-trum within 8 ms. With a sweep width of 200 nm, the resolution achieved is better than 1 nm. If a 128-point spectrum is measured, the required time can be reduced to 4 ms. An essential limitation in decreasing the time of recording a spectrum is the intertia of the galvanometer and its mirror. Its limiting frequency is 500 Hz, so that for measuring times shorter than 2 ms the galvano-meter and its mirror cannot follow the changes in the controlling current. This damps the amplitude of the sweep and the spectrum is shifted with respect to the wavelength. More recently, the same team [111] studied the kinetics and mechanism of various electrode processes by simultaneous coulometric and spectrophotometric measurements.
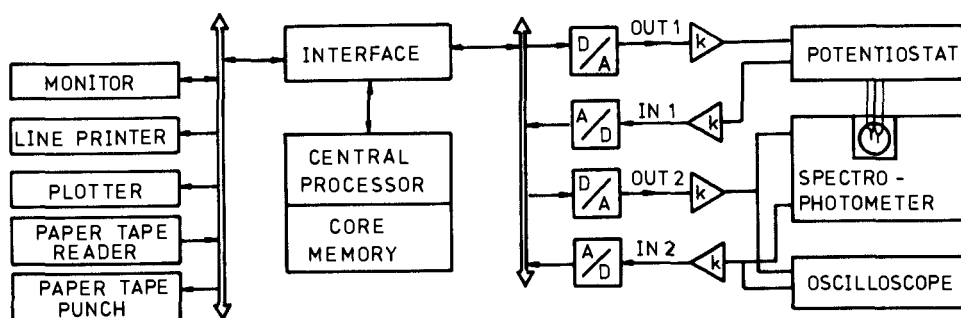


**Fig. 11.23** Block diagram of the computer-controlled spectroelectro-chemical system designed by Strojek *et al.* (Reproduced from [110] with permission of Elsevier).

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# 12

## Automation of analytical instrumentation. III. Chromatographic techniques

### 12.1 INTRODUCTION

Tsvet [1] could hardly have envisaged early this century the future significance of his well-known experiment involving the separation of plant dyes. In fact, since the 1960s, chromatographic processes have occupied a prominent place in both control and research laboratories. Their capability to solve in a brilliant manner problems of all kinds in a variety of fields with socio-economic repercussions has fostered the development of a wide range of commercially available chromatographic instruments. In short, chromatography marked the beginning of a new age in analytical chemistry.

A stringent conception of analytical chemistry propitiated the inclusion of chromatographic processes among separation techniques. However, many modern instrumental analysis textbooks [2,3] have one or several chapters devoted to chromatography. This inclusion is justified only when the chromatographic system concerned possesses some degree of automation and is endowed with a continuous optical or electroanalytical detector coupled on-line to the effluent outlet of the chromatographic column. Such is the case with gas or liquid chromatographs, which can be regarded as instruments rather than apparatuses insofar as they provide information on the sample being subjected to analysis. Other chromatographic modes should be considered as mere separation techniques.

Of the numerous classifications of chromatographic processes proposed to date [4-6], the most interesting from the point of view of automation is that based on the manner in which the process is carried out, which distinguishes two basic types of chromatography, namely:

(a) *Planar chromatography*, in which the separation takes place on a surface, even though there is a third dimension much less significant than the other two. Different types of planar chromatography are distinguished according to the type of support used (paper or a thin layer).

(b) *Column chromatography*, in which the chromatographic material is arranged cylindrically in a variety of manners. It can be divided according to

the state of aggregation of the mobile phase into liquid chromatography and gas chromatography. This is where the term 'chromatograph' falls naturally. Another classification of chromatographic processes complementary to the previous one is based on the near and remote basis of the separation, which obviously depends on the nature of the phases involved. As can be seen from Table 12.1 and for obvious reasons, no type of chromatographic process is based on the use of a gas as a stationary phase.

**TABLE 12.1**

Classification of chromatographic processes

| Phases involved | | Basis of | Name |
|---|---|---|---|
| Mobile | Stationary | the separation | |
| Liquid | Liquid | Partitioning | High-performance liquid chromatography (HPLC) |
| | Solid | Adsorption Ion exchange Exclusion Affinity | High-performance thin-layer chromatography (HPTLC) |
| Gas | Liquid Solid | Partitioning Adsorption | Gas chromatography (GC) |
| Supercritical fluid | Liquid Solid | Partitioning Adsorption | Supercritical fluid chromatography (SFC) |

Although the automation of chromatographic processes involves both planar and column varieties, that of liquid and gas chromatographs is much more important. Nevertheless, automation is also a relevant feature of the recently developed planar chromatographic mode, namely 'high performance thin-layer chromatography' (HPTLC). Because of the parallelism between the technical features of gas chromatography (GC) and high-performance liquid chromatography (HPLC), both techniques are dealt with jointly in this chapter from the point of view of automation, which is described according to the different modules involved.

## 12.2 DEGREES OF AUTOMATION OF A CHROMATOGRAPH

A liquid (HPLC) or gas (GC) chromatograph usually consists of five essen-

tial modules: (1) a system for setting up a flow of the liquid or gaseous mobile phase —the former of which can have a constant or variable composition—; (2) a unit for introduction (injection) of the sample into the mobile phase flow; (3) a separation column housed in a furnace or enclosure whose constant (HPLC, GC) or variable (GC) temperature can be precisely controlled; (4) a continuous detection system reached (destructive) or traversed (non-destructive) by the column effluent, which yields a transient signal upon arrival of each analyte-solute; (5) a data acquisition and processing system. The use of x-t recorders is now outdated; in fact, they have been superseded by electronic integration systems, which, in addition to providing a recording of the chromatogram, supply qualitative and quantitative information on the sample.

The scheme in Fig. 12.1 is illustrative of a chromatograph —whether liquid or gas— and of the different possible degrees of automation. The

**Fig. 12.1** *Scheme showing the modular components of a liquid (HPLC) or gas (GC) chromatograph and illustrating different degrees of automation (from 1 to 4).*

simplest alternative involves the automation of data acquisition and treat-
ment by means of an electronic integrator. This option can be readily extended
to the automation of the control of the furnace temperature, a key feature in
gas chromatography, which usually involves temperature gradients. The sample
introduction system is also liable to automation, and it is this very feature
that is responsible for the term 'automatic' in commercial chromatographs. The
automation of the sampling system may also involve prior steps other than the
sample introduction or injection such as the sample collection and its treat-
ment (preconcentration, interference removal, clean-up, etc), and the use of a
sampler. Finally, there is the possibility of automatically controlling the
system for preparation (mixing) and regulation of the flow of gas or liquid
mobile phase, another key premise to achieving the required degree of separa-
tion and obtaining reproducible results.

The automation of the different stages of a column chromatographic process
involves the use of computerized systems of variable design and complexity ac-
cording to the degree of automation required and the design of the chromato-
graph, which can have a linear or modular conception. There are, however, a
series of more or less general concepts applicable to a large number of chro-
matographs which are commented on below. The relationship between the (micro)
computer and the chromatograph is illustrated in Fig. 12.2.



**Fig. 12.2**  Integral automatic control of a chromatograph.

The acquisition and treatment of data require the use of an electronic in-
tegrator endowed with a series of calculation programs (between 5 and 10) cap-
able of solving different problems and providing qualitative and quantitative
information in addition to the chromatogram. Whenever special programs are
required to deal with chromatographic data from complex samples or data are

generated in huge numbers (use of several detectors, hyphenated methods) at a high rate (diode array detectors), microcomputers satisfactorily replace integrators, which do not offer such a brilliant performance in these situations.

Most of the commercial gas and liquid chromatographs currently available have built-in microprocessors allowing the automatic control of the functioning of one, two or all three essential modules of the chromatograph, namely the continuous generator of the mobile phase, the chromatographic furnace and the continuous detector. In gas chromatography, the furnace temperature is always controlled by a microprocessor, which allows the establishment of linear and exponential gradients, as well as cooling of the furnace between injections. Thermal control in HPLC is less commonplace. Recent gas chromatographs commonly allow the flow-rate of the carrier gas to be controlled via the microprocessor. High-performance liquid chromatographs use dedicated microprocessors for establishing linear or exponential gradients of the composition of the mobile phase by controlling two or more high- or low-pressure pumps —in integrated designs, the microprocessor can be built-in and control other functions. The automation of the sample introduction can be performed as such or through a sample turntable, under the control of a microcomputer in either case. The continuous detection system of advanced liquid chromatographs can also be accomplished by using a microprocessor.

As stated in Chapter 2, the overall automation of a laboratory or a complex control system can be approached in three ways depending on whether dedicated, centralized or hierarchical configurations are involved. The last option is also the most advisable: the sample 'circulates' through different instruments with built-in microprocessors that acquire and process data, which are then sent to the central computer for integration and storage. Liquid and gas chromatographs are key pieces of these configurations, marketed by major firms such as Hewlett-Packard (LABSAM and LABQUEST systems) and Perkin-Elmer (LIMS). The functioning of the different instruments can also be modified from the central computer via each dedicated microprocessor. A total interface approach to GC networking and communication has been reported [7].

## 12.3 MODULAR AUTOMATION OF A CHROMATOGRAPH

The reduction or elimination of human intervention in the operation of a chromatograph (HPLC, GC) should be approached through the automation of its modular components, described in the preceding section. This can be achieved with the aid of a single (integrated design) or several dedicated microprocessors (coordinated or not). Below are described specific aspects of the automation of each modular element of gas and liquid chromatographs.

**12.3.1 System for preparation and control of the mobile phase**

The design of this module naturally varies with the nature of the mobile phase. The use of pressurized gases makes propelling systems unnecessary in gas chromatography which, however, calls for the use of highly precise systems for the measurement and regulation of the flow-rate. Liquid chromatography has stricter requirements: it demands the establishment of highly precise liquid flow pulses to traverse the highly compact chromatographic columns used; the flow-rate must be constant and variable at will and the composition of the mobile phase should also be variable during the process. Automation in this context is much more important in HPLC than it is in GC.

In any case, there are two possible approaches from the point of view of automation:

(a) Selection of parameters characterizing the chromatographic mobile phase such as the pressure, flow-rate and  use of different eluents with different degrees of mixing.

(b) Control of the parameters (usually the flow-rate and pressure), which requires the use of measurement systems that generate an electrical signal acquired by the computer after a suitable transformation. A feedback system allows oscillations to be corrected without human intervention and hence the acquisition tainment of reproducible results without human intervention.

The solvent delivery system (SDS) is a key part of liquid chromatographs. The microprocessor allows the selection of the pressure and flow-rate of the mobile phase by sending appropriate signal to the pump(s). With the aid of transducers, these parameters can be converted to signals sent to the microprocessor in order to establish a feedback system ensuring their constancy and reproducibility of the chromatographic results obtained. When the mobile phase used has a constant composition (isocratic elution), the microprocessor carries out no other task in this module. Automation is indispensable if the composition of the mobile phase has to be varied gradually during the chromatographic process. Such automation can be accomplished with the aid of an electronic programmer controlling the functioning of two high- (Fig. 12.3.1) or low-pressure pumps (Fig. 12.3.2.1) or a proportioning valve (Fig. 12.3.2.2). A microprocessor endows the system with much more versatility insofar as it allows the establishment of gradients precisely matched to the particular analytical problem by direct programming —the programs created for a given purpose, however, can be stored for future use. Moreover, as stated above, the microprocessor can select and regulate the flow-rate and pressure.

The automatic control of the pressure and flow-rate of the carrier gas in gas chromatography can be accomplished by using an electronic flow controller such as the Hewlett-Packard HP 5880A. This module (Fig. 12.4) consists of three essential components:
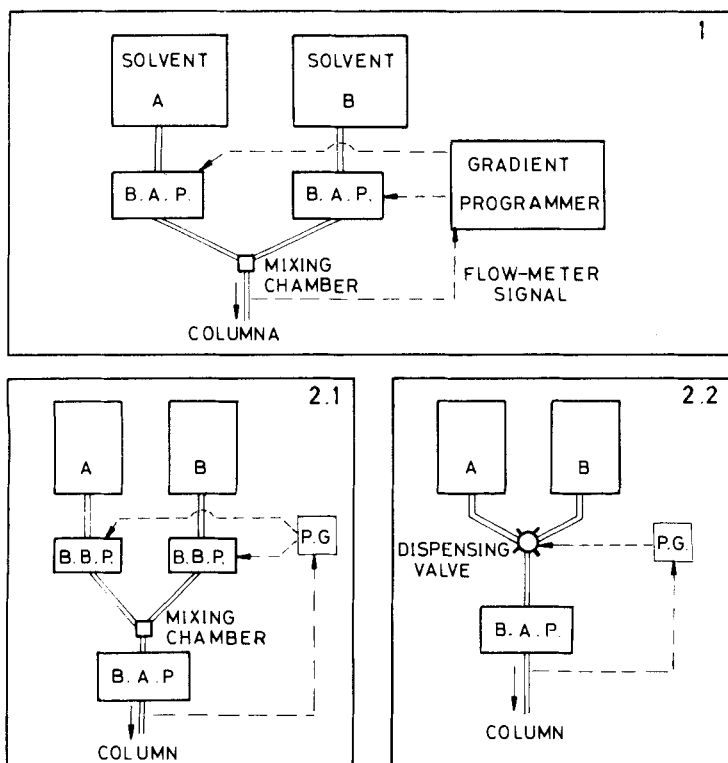
**Fig. 12.3** Use of a mobile phase gradient programmer (simple elec-
tronic system or microprocessor) in liquid chromatography. (1) High-
pressure mixing with the aid of two high-pressure pumps and a mixing
chamber. (2) Low-pressure mixing with the aid of (2.1) two low-pres-
sure pumps and a mixing chamber, and (2.2) a proportioning valve (in
both instances a single high-pressure pump is used).

(a) A pressure sensor based on capacitance measurements and consisting of
a minichamber which is traversed by the carrier gas. Its base is in contact
with one of the plates of a planar condenser connected serially with an in-
ducer to form an oscillator showing the variation of the capacitance through
the alteration of the distance between the plates.

(b) A system with two valves on each side of the sensor, which is located
between the carrier gas source and the injection system. The first is a stop-
go pump while the second is a two-way pump and allows the pressure to be con-
trolled.

(c) A dedicated microprocessor acquiring the signals from the micropro-
cessor housed in the chromatograph and setting the pressure and flow-rate. It

A)



Inlet/Outlet tube

Sensor volume

Moving plate

Fixed plate

Oscillator

B)



Duty Cycle valve

Two way valve

Source

Injection Port

Sensor

Oscillator

Heated Zone

Microprocessor

C)



GC Microprocessor

Setpoint P,F          Actual P,F

Actual P,F

Local Controller Microprocessor

Pressure traducer

Valve actuator

Duty cycle time
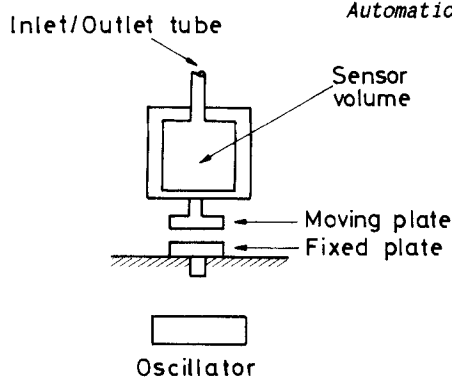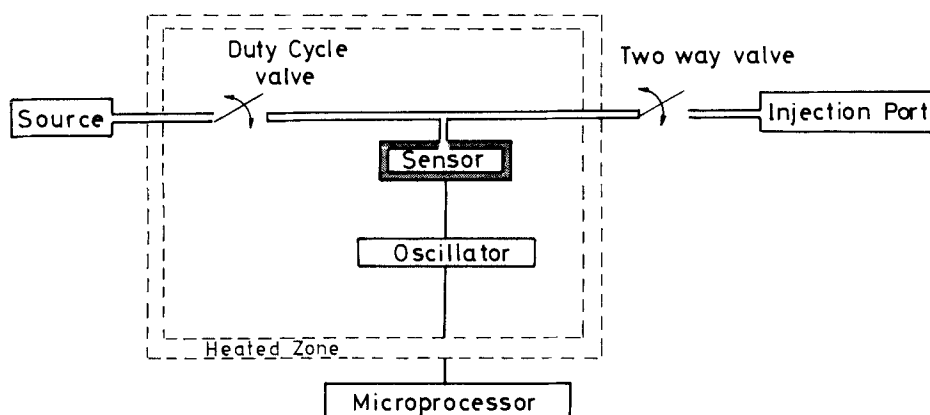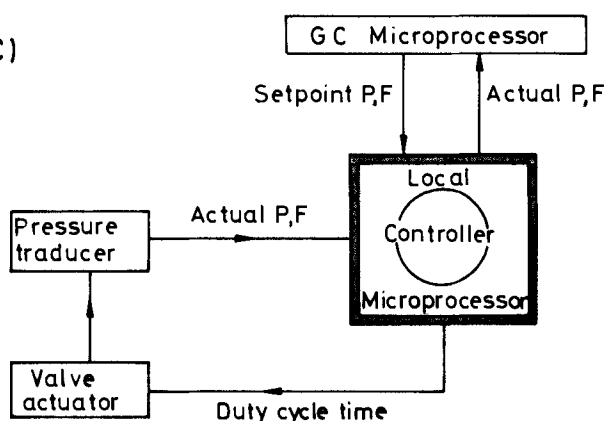
**Fig. 12.4** Electronic flow controller (EFC) in a gas chromatograph. (a) Capacity pressure transducer. (b) Pneumatics of the EFC. (c) Control loop regulation by means of two microprocessors. (Courtesy of Hewlett-Packard).

actuates the pumps and continuously measures the pressure in the sensor and sends real pressure and flow-rate data to the microprocessor.

### 12.3.2 Sample injection

Manual injection into a liquid chromatograph is usually performed with the aid of a rotary valve (generally one manufactured by Rheodyne or Valco), similar to those used in FIA, but more sophisticated and expensive on account of their stricter pressure requirements. The injection operation can be readily automated by coupling a synchronous motor alternately switching the valves between their two positions. This can be controlled via a button or the microprocessor This alternative is of limited versatility as it does not allow programming of the injection valve, because this depends on the loop used, so that any change can only be introduced by manually replacing the loop.

The complete automation of the introduction of liquid samples in HPLC is accomplished with the aid of modules commonly referred to as auto-injectors. The sample is placed in a vial in the simplest case; however, in the most usual situation, a set of samples are placed in the vials of a sampler in order to be able to perform 50-200 chromatographic determinations in a sequential manner and introduce standards periodically. A dedicated microprocessor allows the introduction of variable sample volumes, performing repeated injections of the same sample in order to check the reproducibility, sending a signal to the data acquisition and treatment system to mark the instant at which injection is started (t), etc. The functioning of the autoinjection module of the Hewlett-Packard HP 1090 liquid chromatograph is illustrated schematically in Fig. 12.5. It consists of three parts: (a) a sampling unit composed of a steel needle piercing the rubber or silicone-rubber septum of the vial and submerging in the liquid sample; (b) a volume meter consisting of a suction syringe actuated by a highly precise stepper motor allowing selection of the injection volume; (c) an electrically activated rotary valve whose two positions allow the mobile phase flow passing through the module not to be interrupted and the sample volume taken to be swept to the chromatographic column. The functioning of this module, illustrated in Fig. 12.5, involves three stages:

(a) In the 'normal' (without sample) position, there is circulation between the solvent delivery system (SDS) and the chromatographic column through the sampling unit, while the measurement unit is disconnected.

(b) In the loading position, the sample vial is introduced and penetrated by the needle; the valve is switched and the syringe withdraws a given sample volume. The mobile phase passes directly to the column through the valve.

(c) In the injection position, technically coincident with the normal po-

sition, once the sample vial has been mechanically removed from the suction
position, the aspiration tip is connected to the valve, which is switched back
to the starting position.
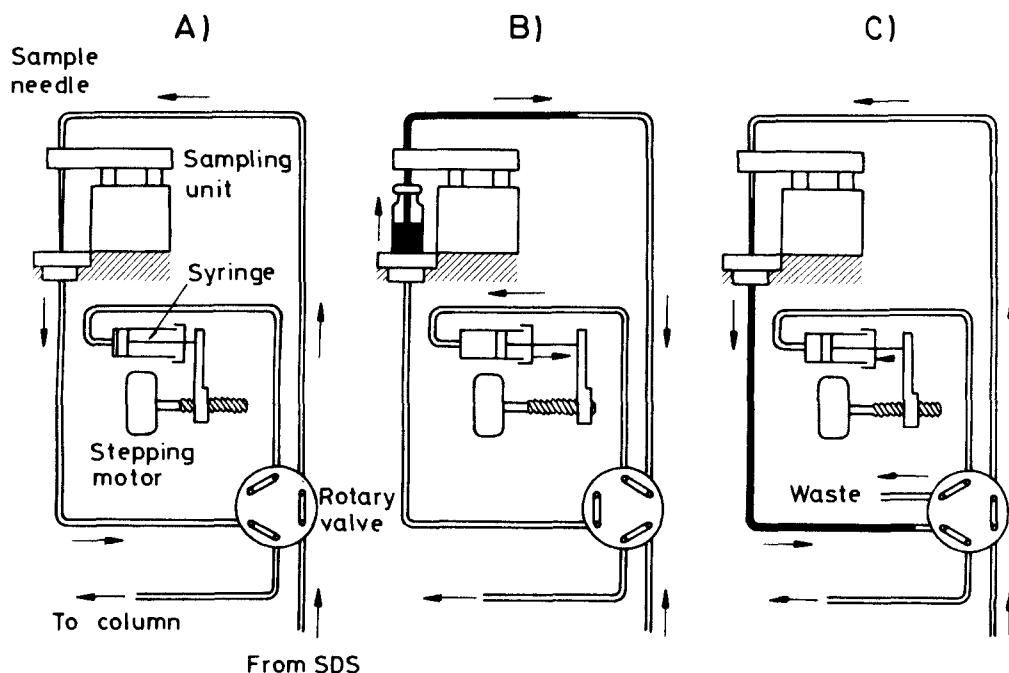


**Fig. 12.5** Functioning of the auto-injection module of the Hewlett-Packard HP 1090 liquid chromatograph. (a) Normal position. (b) Loading position. (c) Injection/normal position. (Courtesy of Hewlett-Packard).

In this configuration, the aspirated sample volume is swept to the chromatographic column. The syringe is activated to pour its contents to waste, thereby avoiding carry-over between samples.

The automation of the introduction of liquid samples in gas chromatography does not differ substantially from that described above for liquid chromatography, although the nature of the mobile phase calls for a slightly different instrumental design. There are several commercially available models, the commonest of which have no sample turntable.

### 12.3.3 Sample collection and treatment

As demonstrated in the first few chapters, both operations are difficult to distinguish in practice as they are carried out in a sequential or simul-

taneous fashion by means of a system depending or not on the chromatograph, and which may function on-line or off-line with this. The latter possibility is the commoner, while the former is the case with continuous separation methods. However, this distinction is not strict as some methodologies may use both alternatives.

In Fig. 12.6 is depicted a scheme showing the most frequent connections established between the sample and the injection module of the chromatograph. The sample can be injected manually (option 1) or by means of a simplified automatic system, i.e. one without a sample turntable. The second possibility (option 2) involves introducing the samples into the vials of a sample turntable incorporated into an auto-injection system specially designed for HPLC or GC. The third alternative (option 3) requires a sample collection system coupled off-line to the chromatograph. Such is the case with the collection of air and water samples at locations a long distance from the laboratory; once taken, they can be pretreated manually or be introduced into the chromatograph without preparation, either directly or through a sample turntable. Option 4 involves the continuous introduction of the sample into a treatment (clean-up, preconcentration) system connected to the chromatograph injector directly or via a sampler.
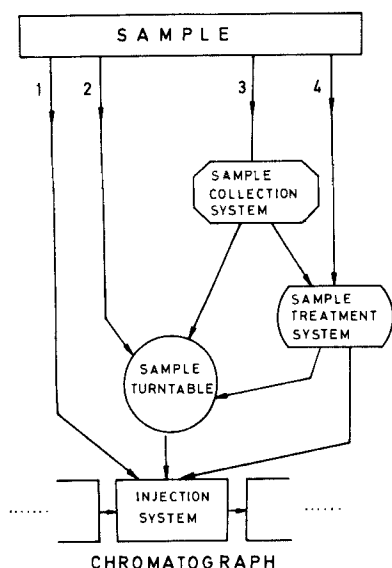


**Fig. 12.6** General manners of connecting the sample and the chromatographic injection module.

The chief objectives of sample pretreatment in chromatography are the same as those of the separation techniques used with any analytical methodology, namely: (a) preconcentration of excessively dilute analytes; (b) sample cleanup, i.e. interference removal; (c) preservation of stored samples prior to their use in the chromatographic stage proper; (d) protection of the chromatograph, particularly of its column, from impurities such as suspended matter and compounds of great chemical activity; (e) facilitation of the analytical determination whenever solvent changeover or removal of excess of reagent are required in a derivatization technique.

Some of the variety of techniques described in the literature have resulted in the commercialization of modules independent of the chromatograph and providing it with different degrees of automation. Such modules are based on extraction (both liquid–liquid and solid–liquid), sorption (adsorption, ion exchange), vaporization, filtration (simple or through molecular sieves) or dialysis processes, or on chemical derivatization techniques. Some of these preliminary operations are better suited to HPLC, others to GC and the remainder equally to both. Only those involving the reduction of human intervention to some extent are described here. This is a wide topic, so a comprehensive treatment is beyond the scope of this book. Below are described some representative examples of both HPLC and GC. Many of the systems described are based on the continuous separation systems dealt with in Chapter 4, devoted to the automation of sample treatment. The foundation of continuous and segmented flow analysers plays a major role in this context.

The earliest attempts at the automation of sample pretreatment in HPLC were made by Technicon who, in the mid-1970s, and taking advantage of their experience with automatic air-segmented continuous systems, developed an interesting series of assemblies designed generically as FAST-LC (an acronym for Fully Automated Sample Treatment for Liquid Chromatography), capable of automatically performing a series of unit operations (solid sampling, extraction, filtration, evaporation) of great relevance to sample pretreatment [9–11]. In Fig. 12.7 are illustrated two examples of pharmaceutical and clinical interest. The first of such systems [12] was conceived for the determination of fat-soluble vitamins (A, D, E) in pharmaceutical tablets at a rate of 2–10 samples per hour, depending on the sample matrix. It combines automatic solid–liquid extraction with solvent changeover in the determination of theophylline and four anticonvulsants and their metabolites in serum [13]. The sample is aspirated and mixed with a buffer containing an internal standard. The resulting flow is mixed with an organic solvent (chloroform–isopropyl alcohol) into which are extracted the drugs and their derivatives in the corresponding coil. The organic phase, once separated from the aqueous phase, is too non-polar for

the extract to be injected on to the reversed–phase column and therefore makes a change of the solvent necessary. This operation is performed continuously in the so–called 'evaporation–to–dryness module' (EDM), patented by Technicon. The organic phase drips on to a Teflon conveyor belt, which drives it to a vacuum zone where it is evaporated, leaving a deposit of the analytes. A stream of a suitable mobile phase (water–methanol–isopropyl alcohol) is intro-duced at the end of the module in order to selectively dissolve the analytes, which are swept to the injector of a liquid chromatograph.
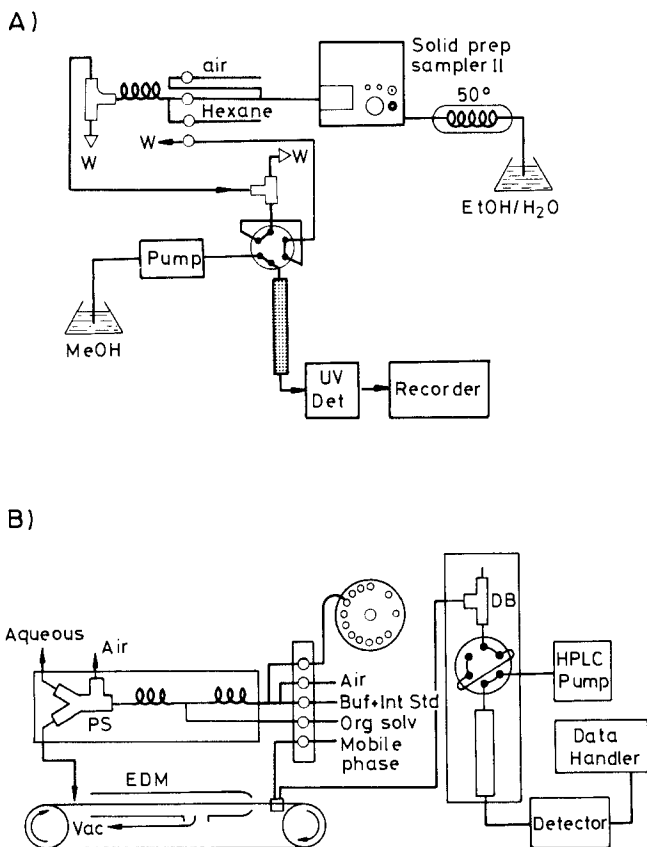
**Fig. 12.7** FAST–LC systems based on continuous air–segmented assem-blies (I). (a) Configuration with pre–column solid–liquid and liquid-liquid extraction. (b) Configuration with liquid–liquid extraction and prior evaporation–dissolution. (Courtesy of Technicon).

In Fig. 12.8 are shown other FAST–LC air–segmented flow assemblies used for sample pretreatment in HPLC. The configuration shown in Fig. 12.8a was de-

signed for the determination of alkaloids [14] and is based on the prior forma-
tion of ion pairs with picric acid which are extracted continuously into a
merging stream of chloroform which finally fills the loop of the injection
valve of a normal-phase chromatographic system. The determination of phenol
pesticides in urine by reversed-phase chromatography [15] requires the precon-
ditioning system depicted in Fig. 12.8b. The sample undergoes a prior hydroly-
tic process and its volatile and non-volatile components are separated by
means of a continuous distillation-condensation system.
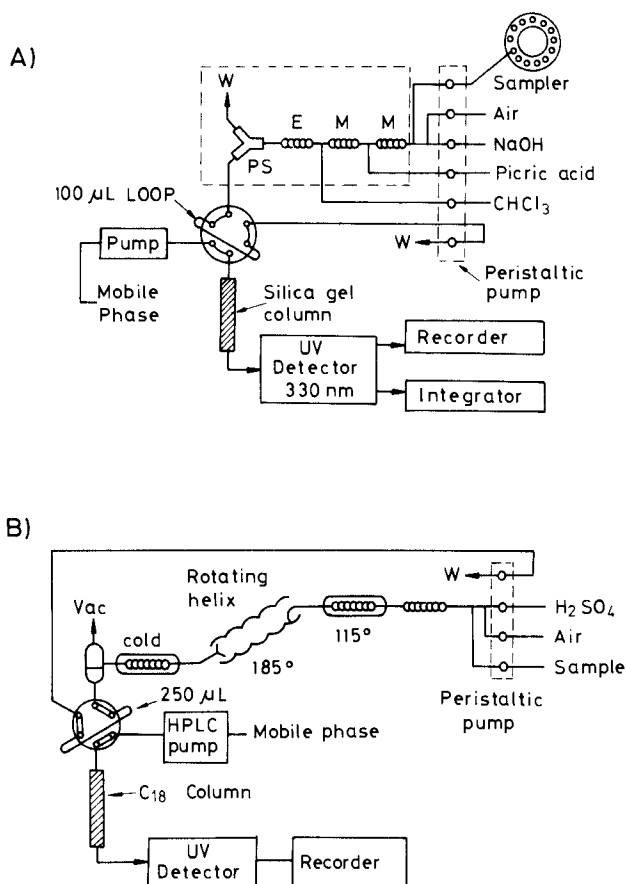


**Fig. 12.8** FAST-LC systems based on continuous air-segmented assem-
blies (II). (a) Configuration with prior liquid-liquid extraction. (b)
Configuration with pre-column hydrolysis, distillation and conden-
sation. (Courtesy of Technicon).

The use of a continuous dialyser for deproteination purposes prior to the

introduction of biological samples in HPLC is more convenient from the point of view of automation, although the low efficiency of the separation product results in serious sensitivity problems, which can be circumvented by performing a prior derivatization. Such is the case with the determination of aminoacids in serum with the set-up proposed by Turnell and Cooper [16] (Fig. 12.9). An iodoacetic acid (IDA) solution of pH 9.5 is mixed with the sample aspirated by the probe. This non-segmented donor stream is passed through the continuous dialyser and then discarded. A derivatizing solution of *o*-phthalaldehyde-2-mercaptoethanol (OPA-MCE) acts as an acceptor stream for the aminoacids and fills the loop of the injection valve of a liquid chromatograph with a gradient of mobile phase [17]. Cooper and Turnell also reported an analogous system including trace preconcentration [18].
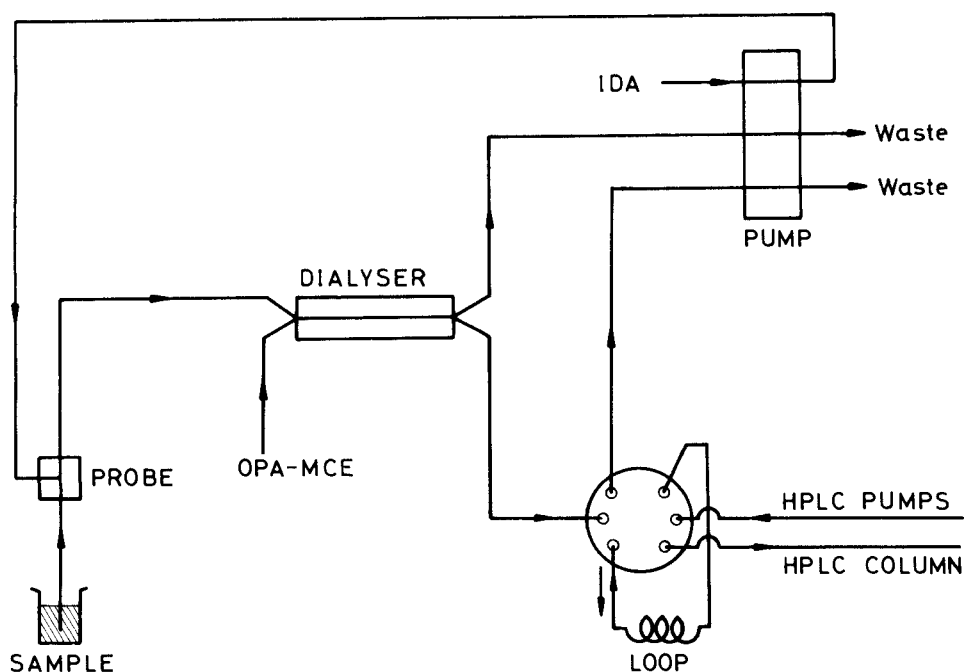


**Fig. 12.9** Continuous pre-column dialysis for the continuous removal of biological samples prior to their introduction into the chromatograph.

Solid-liquid extraction by use of sorbents (silica, alkylsilane-modified silica, alumina, porous polymers with or without ion-exchange groups or carbon materials) contained in a cartridge or a short stainless-steel or glass column is the pretreatment most commonly used at present for some types of samples

and analytes of great socio-economic interest. The extractors used for this
purpose are small (length 2-10 mm, I.D. 2-4.6 mm) and are packed with rela-
tively large particles (25-60 µm) with stainless-steel sieves instead of por-
ous frits and connected to relatively wide-bore capillaries (0.5 mm I.D.).
They can operate in two ways: (a) off-line with the chromatograph in the sam-
pling, preservation and elution and (b) on-line with the chromatographic
system, so that two easily automated sequential operations allow the retention
(clean-up, enrichment) and elution of the retained analytes to the chromato-
graph injector. This latter option has met with wide acceptance and develop-
ment [19-22]; the columns retaining the sorbent are known as 'pre-columns' and
require the use of multi-way diverting valves for the so-called 'column switch-
ing techniques'.
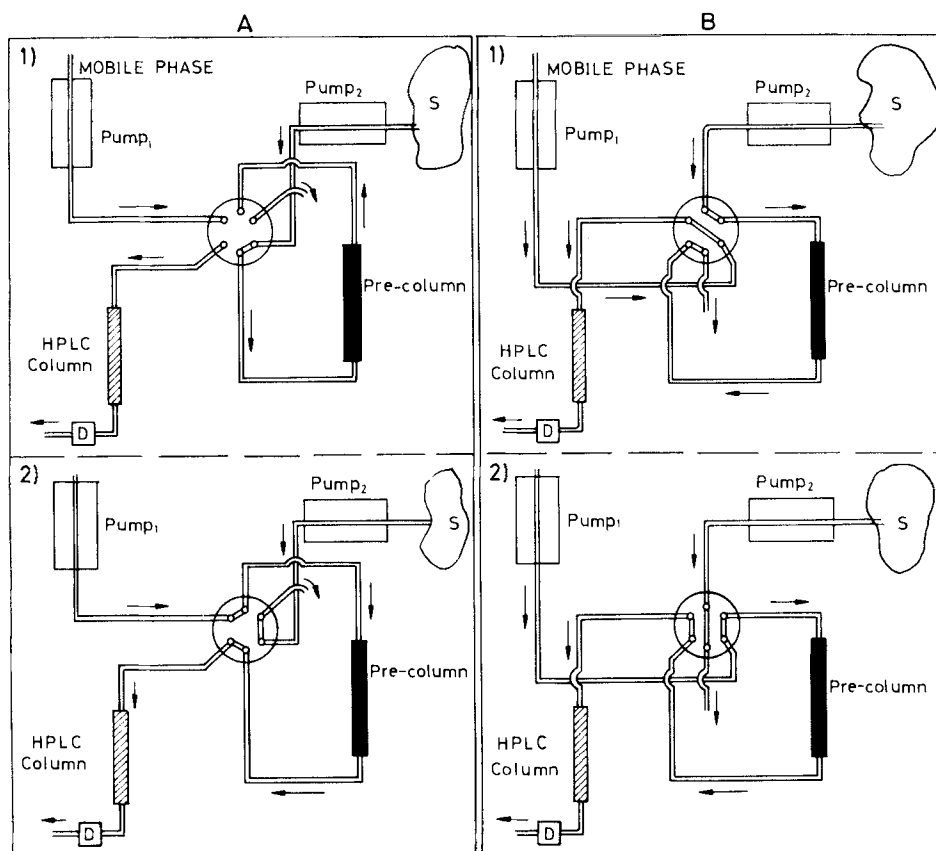


**Fig. 12.10** Schematic diagram of column-switching techniques imple-
mented with a single valve, with (1) preconcentration and (2) elution
in the pre-column in (a) the opposite and (b) the same direction.

In Fig. 12.10 are depicted two configurations using a single rotary valve and differing in the direction of the elution flow. They include two pumps (one of which can be of the low-pressure type and the other a typical HPLC pump) and two columns: the pre-column and the chromatographic column. In the configuration shown in Fig. 12.10a, and in its filling position (1), the aspirated sample stream flows upstream through the precolumn and is led to waste after passing twice through the valve —this is also traversed by the chromatographic mobile phase in passing directly on to the separation column. After a measured sample volume (1 mL to 1L) has passed, the valve is switched to another position by a pneumatic or electric system and the mobile phase passes through the pre-column downstream, rapidly eluting the retained analytes and driving them to the chromatographic column. Pump 2 can then continue to work while the sample is sent to waste as another sample is introduced into the system or this is stopped by switching the valve. In fact, the pre-column can be considered to be included in the loop of a typical injection valve. The second possibility, illustrated in Fig. 12.10b, involves upstream elution (i.e. the eluent is passed in the same direction as the sample). The valve connections are slightly different, while the components used are the same as in the previous case.

Figure 12.11 shows three configurations based on the use of two rotary valves which are activated simultaneously and used for different purposes. The configuration in Fig. 12.11a measures a sample volume injected through the first valve and performs the clean-up in the second valve, which has its pre-column in the corresponding loop. The configuration in Fig. 12.11b is similar to the previous one, but the connections have been altered so that the pre-column elution takes place in the same direction as that of circulation of the sample. It is used chiefly for preconcentration purposes. The configuration in Fig. 12.11a was designed to increase the sampling frequency by using two pre-columns. The sample is injected by means of another prior valve or is not injected if a large volume is to be passed. The sample is first concentrated on a pre-column and then backflushed into the analytical column. The second precolumn is re-equilibrated with the solvent used for enrichment. The two pre-columns alternate in their duty cycle when connected to the analytical column.

The automation of the use of disposable cartridges for solid–liquid extraction connected directly with a liquid chromatograph poses major techical problems which are, however, lessened by robotics. Although several partly automatic systems have been described [23-26], there is only one really completely automated system, which was reported recently [27]. It is an automated cartridge exchange module combined with a low-cost purge pump, a solvent selec-
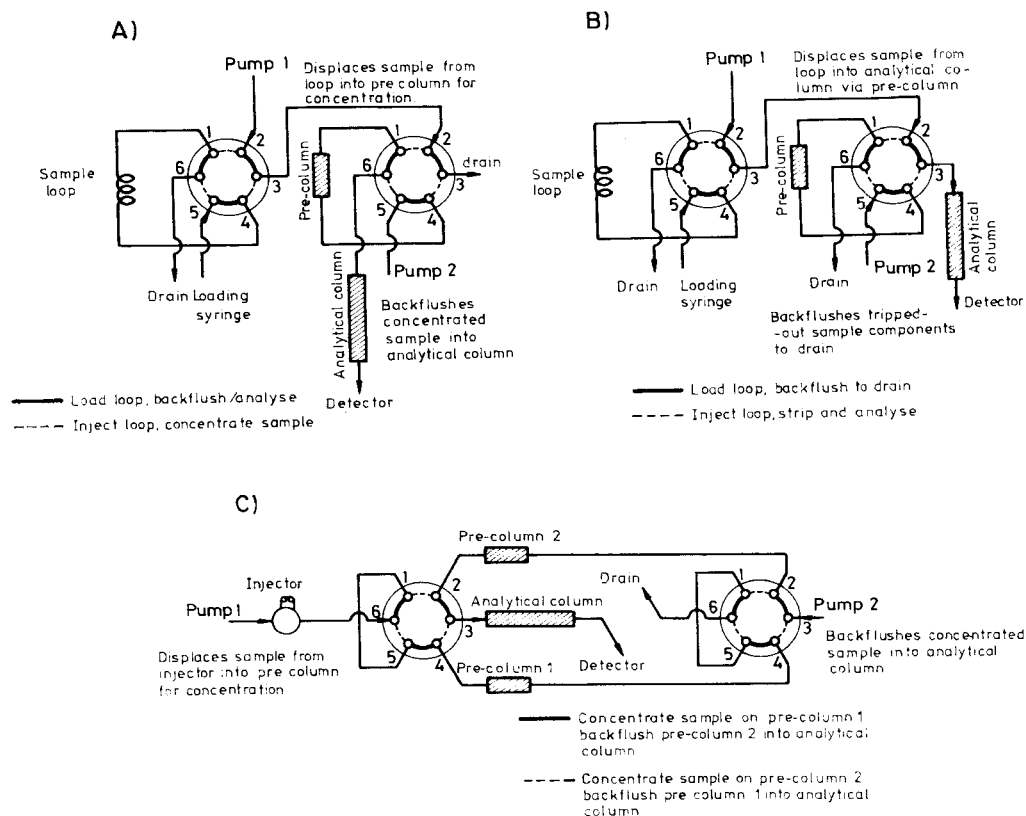
**Fig. 12.11** Column switching techniques with two selection valves which operate simultaneously. (a) For injection/enrichment; (b) for injection/clean-up and (c) for alternating pre-column enrichment. (Courtesy of Rheodyne Inc.).

tion valve (S) and an autosampler which constitutes a fully automated sample handling system for HPLC (Fig. 12.12a). Two selection valves ($V_1$ and $V_2$) accomplish the sequential retention and elution of the sample in each cartridge (C), which is discarded after the elution. In Fig. 12.12b is shown the automated exchange module. The cartridges (length 10 mm, I.D. 2 mm) are placed in a transport strip, which is in turn placed in a reservoir. The pressure-resistant cartridge is pneumatically mounted and connected to a high-pressure switching valve. The self-adjusting transport mechanism is depicted in greater detail in Fig. 12.12c. Unattended operation of the system is possible in the determination of anti-epileptic drugs in serum, an anticancer drug in plasma, barbiturates in urine, phenylurea herbicides in river water and caffeine in a soft drink, among others.

The automation of sample collection and treatment in gas chromatography has had a less extensive development than in HPLC. Some of the systems described above can be used in GC by introducing slight modifications if liquid samples are to be used. Thus, a continuous unsegmented liquid-liquid extraction system was recently developed for the determination of water pollutants [28]. Below are discussed two commercial systems as examples of automation prior to introduction of the sample into a gas chromatograph in dealing with two analytical problems: control of environmental pollution and analysis for volatile compounds in solid or semi-solid samples.

Perkin-Elmer have developed an automated thermal desorption system (ATD 50) which allows the determination of air pollutants by GC. Samples are collected *in situ* by means of portable suction pumps connected to adsorption tubes through which a given volume of air is passed. Once the sample has been taken off-line, the adsorption tubes are introduced into a sampling module connected to the chromatograph. The sample is desorbed and transferred to the chromatograph by the automatic rise of a tube whose heating rate can be programmed and which is inserted into a low- or high-pressure flow system. Direct sweeping of the desorbed compounds to the chromatographic system results in undesirable effects such as band broadening and consequent overlap of the chromatogram peaks. By using a second retention system consisting of a suitably cooled narrow-bore tube, the analytes are again preconcentrated on its wall. Sharp heating of the tube allows the analytes to be swept through a thermally controlled line. The sample introduction system is controlled by a microcomputer which permits (a) control of the sampling unit, (b) control of the valves, (c) selection and measurement of the pressure and flow-rate of the carrier gas and (d) thermal control of the different elements.

Head space gas chromatography (HSGC) is an interesting alternative to the automation of sample introduction in gas chromatography [29]. It relies on the
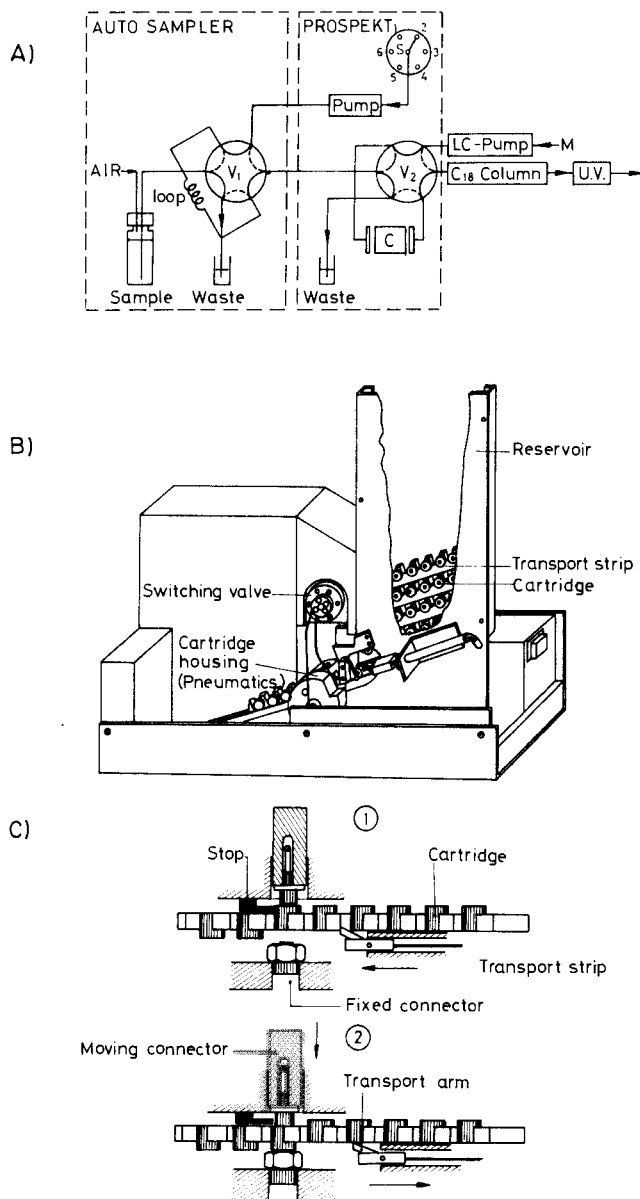
**Fig. 12.12** Fully automated sample handling system for HPLC based on the use of disposable cartridges. (a) General scheme of the configuration, which includes an automated sample handling system (PROSPEKT). (b) Automated cartridge exchange module. (c) Self-adjusting cartridge mechanism: c.1, no connection, allowing cartridge transport; c.2, closed, leak-tight connection with the switching valve. (Reproduced from [27] with permission of Elsevier).

selective volatility of the components of a solid-liquid sample which is suitably heated in a closed vessel. The carrier gas sweeps the volatile components into the column, thereby effecting a prior separation and avoiding the introduction of volatile compounds, dissolution and other preliminary operations. This alternative can be implemented on a number of designs, many of which, however, are difficult to automate. There are also a variety of commercially available automatic HSGC modules, of which the Perkin-Elmer HS-100 system is a representative example. In this, the samples, previously weighed or measured, are placed in vials closed by means of different types of septa according to the nature of the sample and analytes. The vials are placed on a non-circular sample turntable at room temperature, which is thermostated prior to introduction of each volatilized sample into the vial's headspace. Temperature control is mandatory in this stage, the attainment of thermal homogeneity occasionally being accelerated by use of a magnetic stirring system —reproducible results are otherwise difficult to obtain. The collection of the volatile fraction and its transfer to the chromatograph are depicted schematically in Fig. 12.13. A special injection needle through which the carrier gas is circulated is introduced into the vial through the septum, which allows the volatile fraction to be swept. In the thermostating position (Fig. 12.13a), the needle is at its highest position and the carrier gas is led directly to the chromatographic column through the solenoid valve ($V_1$), although a fraction of it circulates through the cylinder housing the needle and is sent to waste through valve $V_2$, which is thus flushed. As the introduction step is started (Fig. 12.13b), the needle penetrates the vial through the septum, the carrier gas flowing through $V_1$ bifurcates to the inside of the vial and the column and its pressure is equilibrated with that found at the head of the chromatographic column. In the transfer or injection of the volatile components of the sample (Fig. 12.13c), valve $V_1$ halts the passage of carrier gas for a strictly controlled time. The vial momentarily becomes a carrier gas reservoir and the analytes are transferred to the column. Once the injection period has finished, valve $V_1$ is again opened, allowing the carrier gas flow to circulate as the needle is raised and a new vial is placed in the introduction position. The automatic control of the different stages results in a precision of about 0.1%.

### 12.3.4 Column furnace

The control of the temperature of the chromatographic column is a key factor both in facilitating the separation and in achieving reproducible results. In fact, the influence of the temperature is much greater in GC than in HPLC. The accomplishment of efficient separations in GC requires not only keeping a
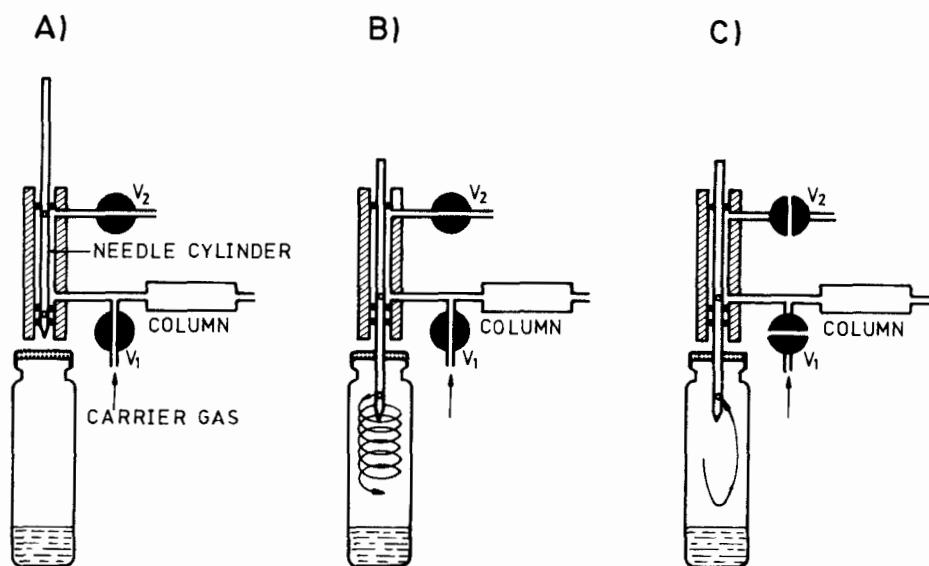
**Fig. 12.13** Automated head space gas chromatograph. (a) Thermostating position. (b) Introduction of gas carrier into the vial. (c) Injection position. (Courtesy of Perkin Elmer).

constant temperature, but the capability to vary it —whether linearly, exponentially or in steps— in the course of the chromatographic process. In addition, a fast cooling system that allows the next injection to be performed without delay —which would detract from the sampling frequency— should be available. Built-in microprocessors, now a common element in commercially available instruments, allow the precise programming of both the initial and final temperature and its variation. A mechanical system electrically controlled via the microprocessor allows the furnace door to be opened and shut and a fan to be started to accelerate cooling. Once the initial temperature is reached again, the microprocessor prompts the user to perform the next injec-

tion through the display or sends a signal to the automatic injection system, depending on the case.

Work in HPLC is generally carried out at a constant temperature usually lower than those typically used in GC. Hence, the automation of the thermal control of the column compartment is fairly infrequent because, in addition, the controlled variation of the temperature is much less efficient than in GC. A straightforward self-contained electric thermostating system is more than adequate for most separations in liquid chromatography.

### 12.3.5 Post-column derivatization systems

Unlike pre-column derivatization, the post-column mode is currently implemented with a series of configurations coupled on-line to the chromatographic column and connected directly to the continuous detector. The off-line alternative is of little practical use today. Hence, there is a significant reduction of human intervention in this context.

Continuous post-column derivatization has reached a considerably lower degree of development in gas chromatography than it has in HPLC owing to the difficulty in carrying out gas-phase reaction on the one hand and to the fact that the performance of GC detectors exceeds that of typical HPLC detectors in sensitivity, response, etc.

Post-column derivatization is widely used in liquid chromatography [30, 31], the potential of which is thereby increased. It affords a number of objectives: (a) indirect improvement of the analyte's intrinsic sensitivity in a given detector (the resultant reaction product absorbs or emits more intense fluorescence than the parent analyte isolated in the column, even after preconcentration); (2) facilitating detection through removal of the excess of derivatizing reagent or thanks to the detector's blindness to the separated analytes; (c) providing an identification test for one or several of the analytes in a complex mixture.

Derivatization reactions are as diverse as the nature of the target analytes can be, hence the large variety of post-column reaction detectors available, the commonest types of which are depicted in Fig. 12.14. Initially, these can be divided according to whether they have (A and C) or do not have (B) an additional pumping system. The reagent can be introduced in two ways: (a) it can be already in the mobile phase and be subject to a physical or physico-chemical change giving rise to the derivatizing reaction in the detector; (b) it can be introduced in a continuous fashion by means of a flow established by an additional pump —this can be implemented with (C) or without (A) a continuous liquid-liquid extraction system using a stream of immiscible organic phase. Thus, every post-column reaction-detection system should consist
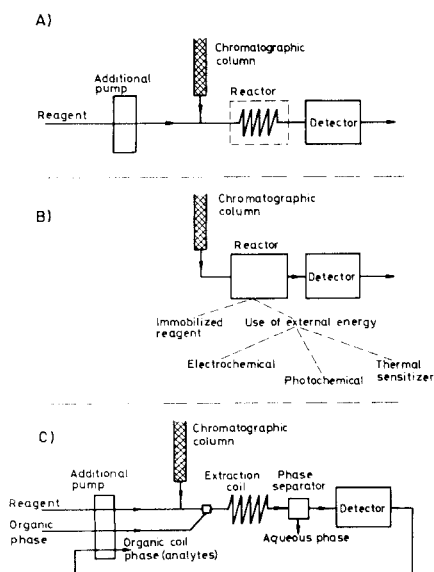
**Fig. 12.14** Post-column reaction detectors most commonly used in HPLC. (a) Pumping of a reagent stream mixing with the chromatographic effluent. (b) Additional pumping systems. (c) Assembly with continuous liquid-liquid extraction (reversed-phase liquid chromatography).

of the following essential elements: a reactor and merging points, one or several low- or high-pressure pumps for establishing additional flow(s), a phase separator for liquid-liquid extraction and a debubbler for air-segmented assemblies.

Derivatization reactions are carried out in various types of detectors. Open reactors (straight, coiled, knotted) are used with fast reactions, while packed reactors or air-segmented systems are to be preferred for slower reactions. The use of reactors packed with an active material (enzymatic or non-enzymatic reactor) taking part in the derivatization is becoming more and more frequent. Post-column liquid-liquid extraction is basically used to remove the excess of derivatization reagent which might interfere with the detection of the reaction product.

The insertion of a derivatization module between the column and the detection system in HPLC can be approached in three ways:

(a) The simplest alternative involves constructing a home-made assembly from the different elements referred to above, which are normally commercially available.

(b) A higher degree of automation is achieved by using the derivatization

modules supplied as accessory elements of liquid chromatographs. These modules are flexible for adaptation to different situations.

(c) A third alternative involves designing a complete liquid chromatograph for a specific purpose. An example is the CHROMAKON, manufactured by Kontron, a representative example of a series of instruments known as amino-acid analysers or sequencers. The functioning of the CHROMAKON is illustrated in Fig. 12.15. In essence it is a computer controlled (10 programs, 172 sentences) high-performance liquid chromatograph with post-column derivatization. It features the customary elements: a reservoir for up to eight buffers, two rotary 8-way selecting valves, an automatic injection system (100 µL) with sampler (60 or 120 sampling positions), an ion-exchange chromatographic column in a thermostated enclosure, a post-column reactor submerged in a dry heating system (100°C) to which a solution of developing reagent (ninhydrin) is sent by additional pumping, and a photometric detector measuring absorbances at 440 and 570 nm. The sampling rate is 40 min per protein hydrolysate sample, plus 55 min for the regeneration cycle.

### 12.3.6 Continuous detection systems

The continuous monitoring of the chromatographic effluent is the key aspect in a chromatograph to be categorized as an instrument according to IUPAC's definitions. The automation of the functioning of the detection module is unncessary unless its characteristics are to be changed during its opera-
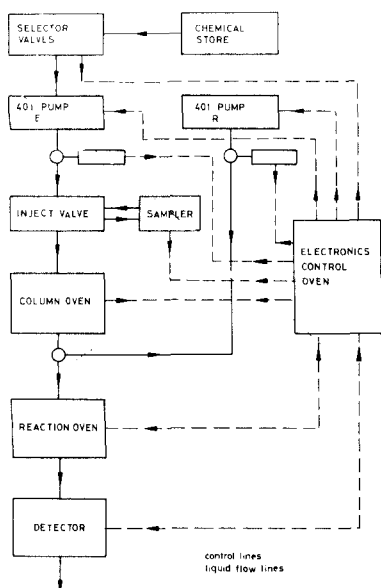


**Fig. 12.15** Schematic diagram of the CHROMAKON 500 amino-acid analyser. (Courtesy of Kontron).

tion or between samples insofar as it works under invariable conditions. This situation is common in the detectors used in gas chromatography (thermal conductivity, flame ionization, electron capture), which require an adequate electronic amplifier but whose functions are constant. Conversely, optical (photometric, fluorimetric) and electroanalytical (basically voltammetric) HPLC detection modules require selection of their operating conditions (wavelength, slit width, applied potential), so they can be automated through a microprocessor, generally built into the liquid chromatograph. In addition to the advantages inherent in the reduction of human intervention, automation offers substantial advantages increasing the quality of chromatographic results.

As an example of the automation of an HPLC detection module we describe here a filter colorimeter (Hewlett-Packard), the optical scheme of  which  is shown in Fig. 12.16a. In addition to the UV-visible light source and the lens system, a disc furnished with peripheral interference filters converts the incident light beam to monochromatic radiation which is split into two beams that are sent to a reference and an analytical photodiode, the latter of which is located after the flow cell. The microprocessor allows the automation of a number of functions:

(a) Selection of the wavelength by appropriate turning of the filter disc (changes are made in less than 1 s by means of a motor).

(b) Selection of the response time, which should be very short (100 ms) for fast peaks and longer (800 ms) for the slower peaks in order to ensure adequate spectral resolution.

(c) Optimization of the signal-to-noise ratio by modifying the current reaching the lamp in accordance with the selected response time.

(d) Changes of the wavelength during the chromatographic process to select the optimum value for each analyte. Figure 12.16b shows the chromatograms obtained in the determination of fat-soluble vitamins by HPLC; when the absorbance is monitored at 340 nm, vitmain A-acetate and vitamin A-palmitate yield the most favourable response, whereas vitamin D gives the best response at 254 nm. To avoid the recording of two chromatograms, the microprocessor changes the wavelength during the chromatographic process.

(e) Changes of the wavelength to ensure a linear response (dilution method). Thus, for a highly concentrated solute, the wavelength selected is far from the absorption maximum, thereby achieving automatic electronic dilution for a given component through a simple change of filter. This allows the simultaneous determination of macro and trace components.

One of the trends in HPLC instrumentation involves the incorporation of image detectors in UV spectroscopy and, in particular, that of diode array photometric systems, whose optical scheme departs from the traditional as can be seen from Fig. 12.17. Thus, they have no monochromator; the light beam im-

A)



B)
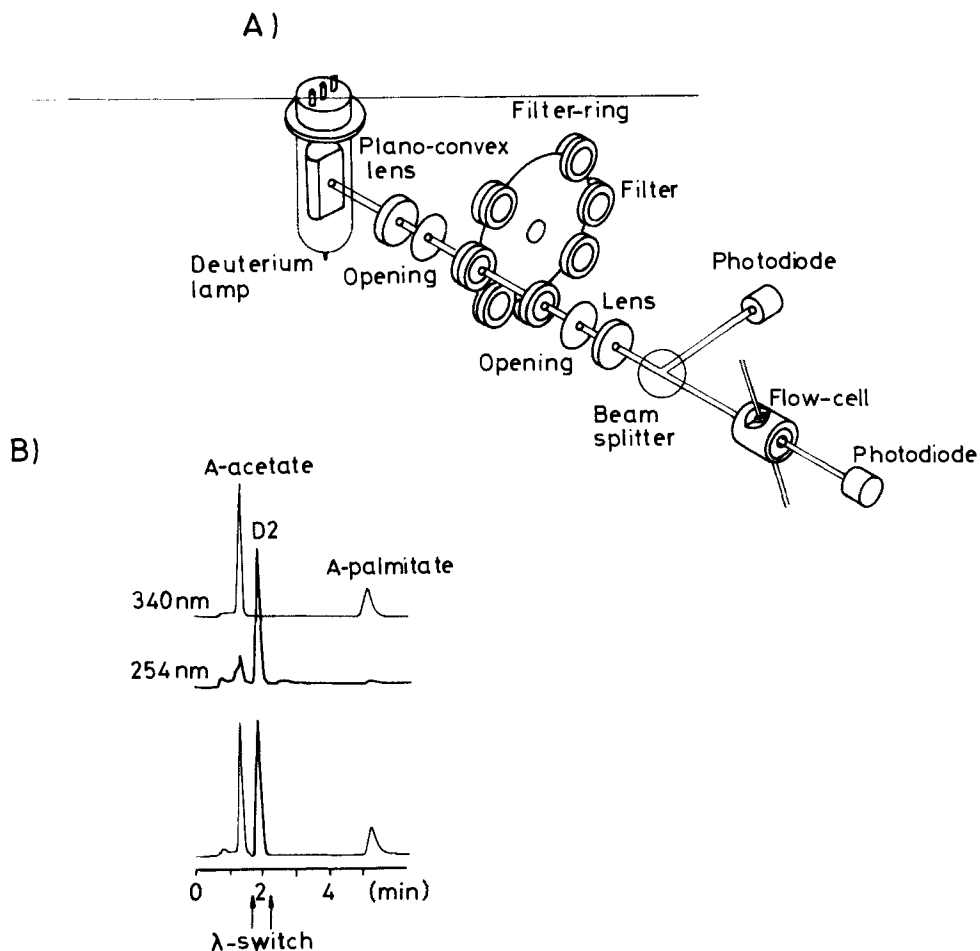


**Fig. 12.16** Automation of the functioning of a colorimetric detector in HPLC. (a) Scheme of the instrument optics. (b) Application of the automatic change of the wavelength during the chromatographic process: determination of fat-soluble vitamins. (Courtesy of Hewlett-Packard).

pinges directly on the flow-cell. A fixed diffraction grating disperses the beam to a diode array (between 400 and 1500), which simultaneously receive the radiation at each wavelength. This configuration endows data acquisition with a high speed, which allows the recording of UV-visible spectra in less than 0.1 s. The dynamics of the hydrodynamic system of a liquid chromatograph are highly compatible with this configuration as it allows the acquisition of absorbance data at many wavelengths in a simultaneous manner, so that the information provided by the detector is much richer than that obtained from an or-

dinary photometric detector. From the scheme in Fig. 12.17 it follows that automation not only hardly affects the optical system, as with the configuration described above, but is also indispensable in order to cope with the large number of rapidly generated data. Thus, the potential of diode array photometers is discussed in the next section, devoted to the last stage of the chromatographic process.
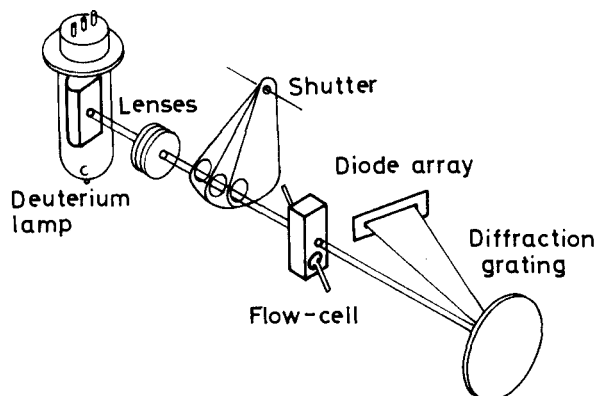


**Fig. 12.17** Scheme of an HPLC diode array photometric detector. (Courtesy of Hewlett-Packard).

### 12.3.7 Data acquisition and treatment

Continuous detection on a chromatograph requires the use of an x–t recorder providing a chromatograph of great overall informative character. However, this is an alternative of little efficiency in practice as the extraction of the qualitative and quantitative analytical information from the chromatogram is time-consuming and prone to errors —occasionally of considerable magnitude. It has therefore understandably fallen into disuse.

The automation of column chromatography started with the module carrying out the last stage of the process. The need to reduce human intervention and augment the precision of the identification and quantitation, together with the increasing efficiency of chromatographs, which are capable of discriminating between a large number of analytes with similar properties, and the occasional demand for high sampling frequencies, make the use of an electronic or computerized system indispensable. Such a system has three functions: (a) acquisition of data, which can be generated in very large numbers or at a high rate; (b) treatment of the chromatogram data for converting them to qualita-

tive and quantitative information; and (c) delivery of results according to the requirements.

There are two general ways of automating this stage. The simpler involves the use of an electronic integrator with 5-15 programs. Commercial integrators are now fairly commonplace in column chromatography. Despite their lack of flexibility, they are useful for most applications. Through a built-in printer they provide both the chromatogram and data such as retention times (whether corrected or not) or the sample composition (based on the measured peak areas), and perform various functions such as calibration and recalibration, cancellation of the solvent signal (peak), resolution of poorly resolved peaks, correction of the baseline, etc.

The use of a microcomputer is a much more flexible option with a greater potential. Compatible computers are being increasingly used in column chromatography. Figure 12.18 depicts the scheme of a system for data acqusition and treatment. The data acquired after amplification and transformation of the continuous signal from the detector can be handled directly by a preselected program and the results are delivered through a printer or plotter. In addition, the data can be stored in the computer's memory and be re-treated if the program initially chosen is inappropriate or be accumulated in order to establish comparisons between samples.
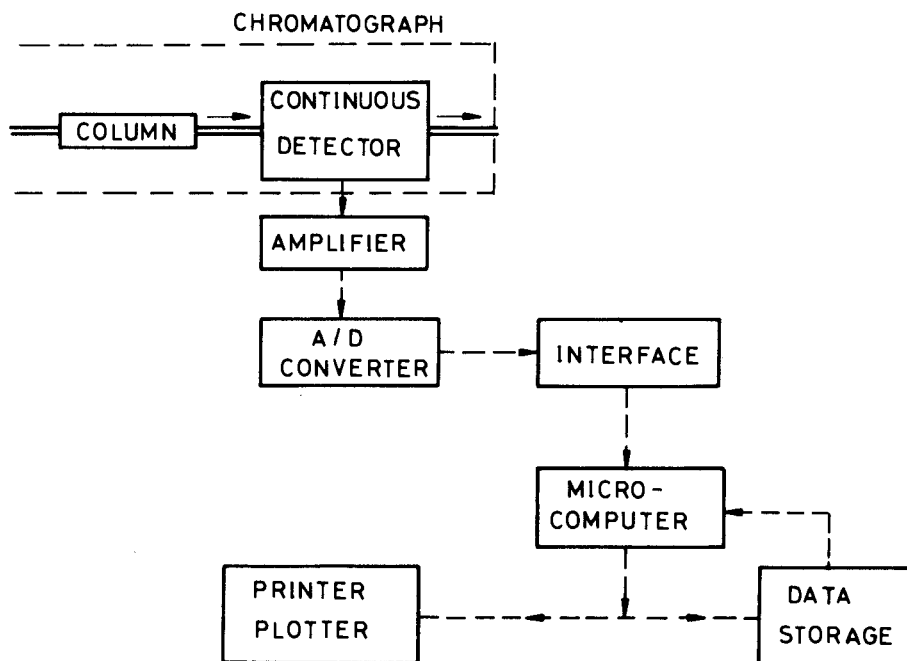


**Fig. 12.18** Use of a microcomputer for the acquisition and treatment of data in column chromatography.

One of the major limitations of the chromatogram provided by a conventional detector is the poor reliability of the identification: retention times are poorly informative in assigning a peak to a given analyte unless methods such as the use of internal standards in identification tests are used. Hence, a current trend in column chromatography involves the use of new, more sophisticated detection systems offering higher levels of information. In addition to the use of two or more detectors (in series or parallel, or integrated) [33], the other two major alternatives demand the use of high-capacity microcomputers —the use of image detectors and instrumental hybridization are commented on below.

As stated above, diode array detectors require the use of a suitable computerized system allowing the acquisition of signals at a high rate in order to reflect the results as required. The use of diode array photometric detectors has resulted in great advances in HPLC [34-40] which have materialized in the commercialization of a variety of modules. Multiplexers are indispensable elements for the virtually simultaneous acquisition of the data from each diode. Figure 12.19a shows the three-dimensional (absorbance-wavelength-time) graphs that can be obtained instead of ordinary chromatograms. Nevertheless, the practical advantages resulting from the simultaneous monitoring of the absorbance at several wavelengths are substantial and can be summarized as follows:

(a) Simultaneous acquisition of several chromatograms (one per wavelength) recorded by using the optimal spectral features for each analyte or group of analytes. An example is the determination of flavonoids (350 nm) and cardenolides (217 nm) in an oleander extract (Fig. 12.19b).

(b) Checking the spectral purity of the chromatographic peaks. If the computer is programmed to acquire the complete spectrum of the chromatographic fluid portion as the solute passes through the flow-cell at three significant moments (e.g. at the peak maximum, at its half-height and on its falling portion), the automatic comparison of these spectra allows one to determine whether a single solute or a mixture is being handled (Fig. 12.19c).

(c) Development of amplification and dilution methods. The acquisition of pairs of absorbance-wavelength data at the maximum of the chromatographic peak allows the manipulation of a large number of data, in contrast with the conventional situation based on the measurement of the absorbance at the maximum of the absorption spectrum (1 in Fig. 12.19d). This allows the sensitivity to be increased by automatic addition of the absorbances at several wavelengths or decreased by using a single absorbance at a wavelength distant from the maximum. Thus, the small peaks yielded by trace components can be amplified, while off-scale peaks can be electronically 'diluted'. For quantitative pur-
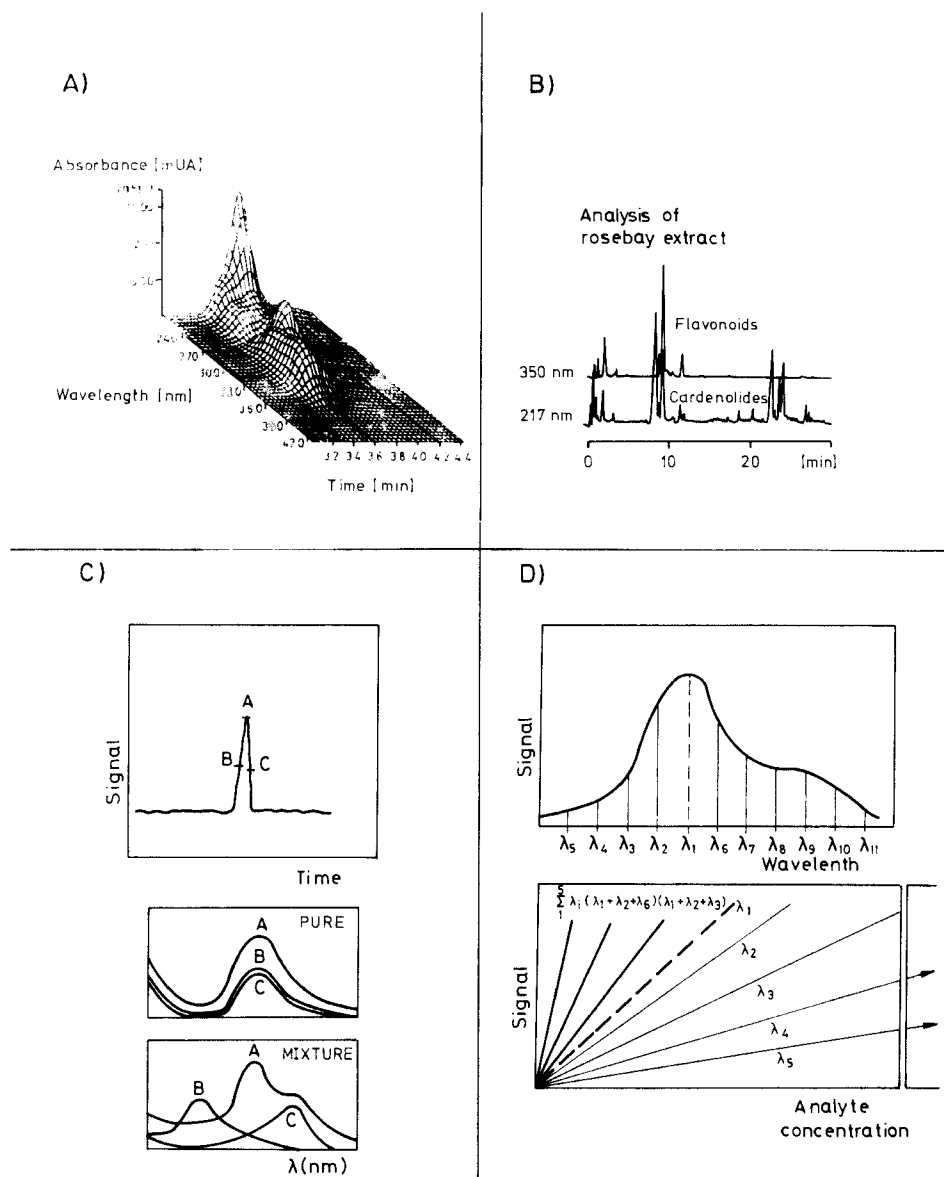
**Fig. 12.19** General possibilities of the use of a diode array detector coupled to a microprocessor in HPLC. (a) Three-dimensional chromatogram. (b) Monitoring at several wavelengths. (c) Checking of the spectral purity of a peak. (d) Manipulation of the sensitivity.

poses, the computer can generate a beam of calibration graphs by use of different standards, graphs which are selected in establishing the sample concentration required for making the minimum possible error.


## 12.4 HYPHENATED METHODS

Instrumental coupling or hybridization [41] is defined as the combination of two or more independent analytical techniques (i.e. techniques which can work autonomously) through an appropriate interface, which generates integral information on the sample composition that is more complete than that achieved with either instrument individually. Despite the large variety of instrumental hybridizations described to date, the most relevant and widely commercialized are those in which one of the instruments used is a gas (GC), liquid (HPLC) or supercritical fluid (SFC) chromatograph. The reason is very simple: they combine the high separation power of chromatographic techniques with the high identification power of spectrometric techniques such as mass spectrometry (MS), Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) and atomic emission (MIP, TCP) or absorption (AAS) spectrometry. In addition, their joint use compensates for the disadvantages of chromatography (unreliable identification) and spectrometric techniques (the need for highly pure analytes).

The key aspects of hyphenated methods are closely related to their automation. The interface or link between the instruments, through which the chromatographic effluent is transferred to the hybridized detector, is generally configured on-line, although it can also operate in an automatic discrete fashion based on the individual entrapment of the analytes. The microprocessor is an indispensable element in instrumental hybridization, where it performs two essential functions: on the one hand, it controls the coordinate operation of both instruments (separative and determinative); on the other, it is the only viable option for dealing appropriately with the avalanche of signals and data generated by each sample introduced into the chromatograph.

In Fig. 12.20 are shown schematically the chief hybridizations possible between  chromatography and various spectrometric techniques. The simplest configuration (Fig. 12.20a) involves the linkage between the column and the spectrometer detection zone via a suitable interface —the information is obtained from the spectrometer only. This configuration is one of the commonest in GC-MS [42-44], HPLC-MS [45], HPLC-plasma emission (ICP, MIP) [46], SFC-MS [47] and HPLC-NMR [48] hybridizations. In the configuration in Fig. 12.20b, the typical non-destructive detector (thermal conductivity in GC and UV-visible in HPLC) of the chromatograph provides an ordinary chromatogram;

A)



B)



C)



D)



E)
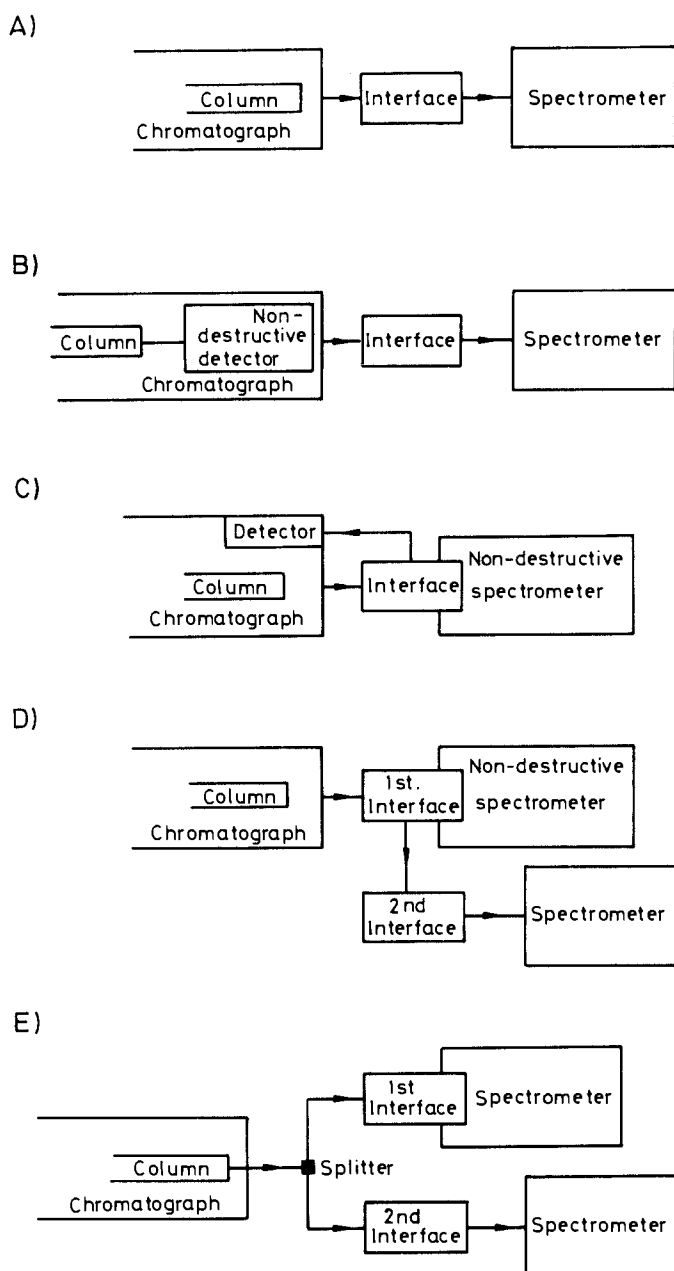


**Fig. 12.20** Schemes of the chief instrumental hybridizations possible between a chromatograph (LC, GC or SFC) and various spectrometers. For simplicity, the microcomputer has been omitted from the schemes.

the peaks are characterized by linking the chromatographic effluent to the spectrometer via a suitable interface. The non-destructive character of the detector may change from the chromatograph to the spectrometer, as shown in the also serial configuration in Fig. 12.20c, in which the effluent enters and leaves the chromatograph through an interface which is usually a flow-cell. Such is the case with GC-FTIR and HPLC-FTIR [49–52].

Triple hybridizations between a chromatograph and two spectrometers of different natures were recently reported. There are two possible configurations: a serial one (Fig. 12.20d) and a parallel one with a flow splitter (Fig. 12.20e). One of the commonest fields of application of these configurations is GC-IR-EM hybridization [53,54], a schematic diagram of which is shown in Fig. 12.21a. As can be seen, the splitting of the flow is not homogeneous, but dependent on the relative sensitivity of the spectrometers. Figure 12.21

A)
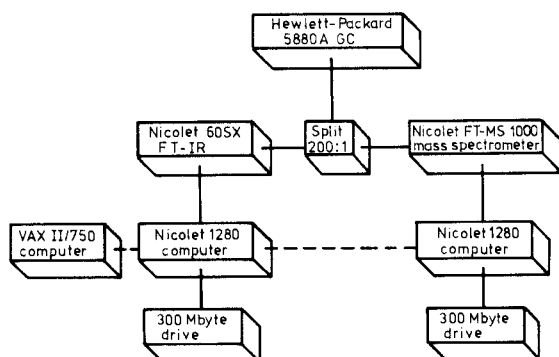


B)
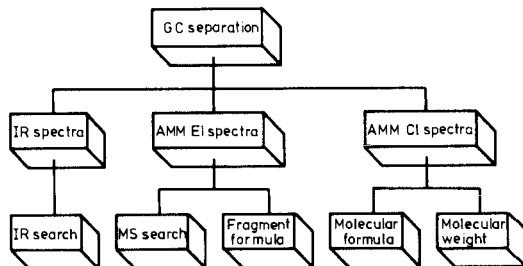


**Fig. 12.21** GC-FTIR-MS hybridization. (a) Scheme of the configuration. (b) Information available from linked accurate mass measurement (AMM) electron ionization (EI)-chemical ionization (CI) and infrared spectra. (Reproduced from [53] with permission of the American Chemical Society).

also shows the information obtained from the integration of the data provided by the IR spectra and the different types of mass spectra obtained for different types of ionization, which are compared with those stored in the computer's library. If there is no coincidence of library search 'hits' (IR/MS), or if the best match is inconsistent with AMM or CI data, no identification is made. This hybrid configuration has been satisfactorily checked with mixtures of up to 45 components.

## 12.5 AUTOMATED OPTIMIZATION OF CHROMATOGRAPHIC SEPARATIONS

The development of a chromatographic procedure for an unknown sample (mixture) requires the selection of a variety of experimental conditions (type and composition of the mobile phase, characteristics of the column and the stationary phase, temperature, flow-rate, pressure, type of gradient, etc.). This problem was traditionally solved in an empirical way and with the aid of the vast literature on similar situations already dealt with. The last few years have seen attempts at the rationalization and automation of the optimization of chromatographic processes which have resulted in interesting systematic approaches of great use. The monographs by Berridge [56], devoted to HPLC, and that by Shoenmakers [57], which deals with both HPLC and GC, represent the most systematic and complete compilations in this field at present.

As a rule, chromatographic optimizations are based on the trial-and-error approach, relying on experimentation, and the basic relationships of chromatographic theory. In HPLC [58-62] is used the well-known expression for the resolution

$$R_s = \frac{1}{4} (\alpha-1) \, N^{1/2} \, \frac{R'}{1+R'}$$

which includes both the selectivity ($\alpha-1$), the efficiency ($N^{1/2}$) and the capacity [$R'/(1+R')$] factor, represented by $\alpha$ (separation factor), $N$ (number of theoretical plates) and $R'$ (mean of the values of the retention or capacity factors of two adjacent peaks, $R'_1$ and $R'_2$). The practical procedure for the determination of $R_s$ is as follows:

(1) A stationary phase, usually in the reversed-phase mode, is chosen.

(2) The composition of the mobile phase is chosen so that the peaks fall at the centre of the chromatogram (by varying $\alpha$ through the percentage of modifier or the pH). If the required separation is not achieved, the optimization is continued as described below.

(3) The selectivity is changed by changing the stationary or mobile phase

(e.g. by replacing the modifier or the reagent yielding the ion-pair) or the chromatographic mode (e.g. to the reversed-phase mode, ion exchange).

The procedure followed in GC is similar, but the column temperature plays a role similar to that of the mobile phase composition in HPLC [63].

The optimization of a chromatographic process usually calls for a large number of experiments which generate a vast amount of data. If it is performed manually (i.e. with the sole intervention of the operator), the procedure is slow and subject to major errors of interpretation, which may shift the search for the optimal conditions in wrong directions and call for an unnecessarily large number of experiments.

The use of computers for optimization purposes has brought about considerable advances as they allow:

(a) Minimization of the number of experiments required.

(b) Acquisition, storage and treatment of the generated data, in addition to drawing useful and reliable conclusions in a short time. This generally involves the use of mapping techniques based on different computational programs. Thus, Snyder's selectivity triangle is frequently used in HPLC for selecting the mobile phase composition.
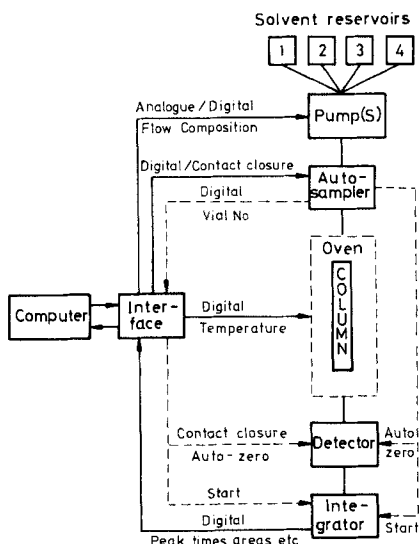


**Fig. 12.22** Scheme of integral automated system for the optimization of separation processes in HPLC showing the essential (solid lines) and subsidiary (dashed lines) communication links. (Reproduced from [56] with permission of John Wiley & Sons).

(c) Programming the functioning of the automated chromatographic modules in order to perform new experiments according to the results previously obtained and the route marked by the computer program. Figure 12.22 shows the scheme of integral automated optimization in HPLC.

The experimental optimization procedures outlined above can be replaced with others based on computer simulations [64,65], which make use of the chromatographic theory and of one or two prior experiments intended to define critical parameters such as the sample, mobile phase, column, temperature, flow-rate and pressure. Simulated chromatograms are obtained for different experimental conditions (column dimensions, particle size, mobile phase composition, flow-rate, temperature, etc.) until the required resolution is achieved. In essence, the procedure is similar to experimental optimization, although the chromatograph functioning is replaced with programming. The information obtained can be checked experimentally or be used for designing new approached to experimental optimization.

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# 13
## Automatic titrators

### 13.1 INTRODUCTION

This chapter presents an overview of commercial and non-commercial titrators excluding flow systems or continuous titrators, which were dealt with in Chapter 7.

Although the number of direct instrumental methods of analysis is growing steadily, titrations are still common practice in routine analyses carried out in many laboratories, probably as result of the lack of sufficiently selective instrumental methods and of the greater sensitivity of automatic titrations. Manual titrations, on the other hand, are time-consuming and the accuracy of the results obtained depends to a great extent on the operator's skill. This is not the case with automatic titrations, of proven efficiency and accuracy [1-3].

A titration usually involves two distinct aspects, namely the control of the performance of the actual titration and the calculation procedure(s). The most relevant advances in both are listed chronologically in Tables 13.1 and 13.2.

The performance of the titration can be controlled in a variety of ways (see Table 13.1): by use of empirical equations for the calculation of $\Delta V$ from preceding titration data points; by use of microprocessors to control volumetric equipment (e.g. in photometric, potentiometric, coulometric titrations) or expand the scope of a given technique; by use of robot stations in implementing laborious manual methods or in handling toxic or hazardous substances; etc. End-point detection is usually based on $\Delta E/\Delta V$ maxima and on first or second derivatives in the case of microprocessor- and microcomputer-controlled processes, respectively. Table 13.2 lists a chronological selection of calculation methods applied to titration curves [46].

Titrators are wet-chemistry analytical instruments comparable in complexity to current process gas chromatographs. They are normally expensive and cannot be run for long periods unattended, though their reliability has reached a point where maintenance efforts are chiefly concerned with the sample system and with ensuring that an adequate supply of reagent is on hand.

**TABLE 13.1** Chronological survey of automatic titration procedures

| | | | | | | |
|---|---|---|---|---|---|---|
| [4] | Empirical eqn. for calcn. of $\Delta V$ from preceding titration data points | Radiometer system | Empirical formula | Inflection point number | 12 | 0.6 |
| [5] | Application of micropr. to control titration | INTEL 8001 CYBA-0 | Constant rate | Max $\Delta pH/\Delta V$ | – | 0.3 |
| [6] | Microcomp.-controlled automatic titrator with automatic sample processing | Mettler system | No information | | – | – |
| [7] | Autom. pot./photom. system, coulom. titrant generation; 3-dimensional plots | PDP DEC-syst.-10 | Constant rate | Overtitration, then calculation | – | |
| [8] | Versatile microcomp. contr. titrator for pot., photom., coulom., etc. titrations | ADD 8080 | Two different constant rates | Max $\Delta E/\Delta V$ | 200 | 0.1 |
| [9] | Microcomp. contr. system for automatic pK determinations | INTEL 8080 | Simplified Christiansen method | | 50 | 0.04 |
| [10] | Microproc.-contr. automatic differential titration | INTEL MCS-80 | Constant $\Delta V$ | Max $\Delta E/\Delta A$ | 30 | 0.7 |
| [11] | Microcomp.-contr. automatic photometric system | INTEL 8080A | Constant | Break point of plot | 500 | 0.6 |
| [12] | Stepwise addition of equal volumes; also suitable for mixtures of polyprotic acids/bases | HP 9835/45 | Constant $\Delta V$ | First deriv. | 25 | |
| [13] | Automatic microcomp.-contr. potentiometric titrator for student demonstrations | ALTAIR 8800B | Two diferent constant rates | $\Delta E/\Delta V$ max. | | 0.1 |
| [14] | Microcomp.-contr. pot. analysis; describes a new computer language: CONVERS | IMSAI 8080 | Christiansen method | Second deriv. | 50 | 0.1-1.0 |
| [15] | Learning method, for strong acids and bases | JOLT system | Determination in learning mode | | | As manual |
| [16] | Fully automated computer-controlled system. $\Delta V$ approximated on the basis of constant $\Delta pH$ | HP 9835/45 | From preceding data | First deriv. | 25 | – |
| [17] | Computer-contr. titration based on systems theory | ZILOG 2.80 VARIAN V76 | Preceding data, feed-back factor | First and second deriv. hyperbolic function | 50 | 0.05 |
| [18] | Robotic sample preparation station. High-class hardware system | APPLE II | Constant rate | First deriv. | 28 | 0.6 |
| [19] | Microcomp.-contr. system for pharmaceutical use (pK determ.) | IM-6100 m.p. | Constant rate | Second deriv. corrected | 100 | 1.0 |

**TABLE 13.2** Chronological selection of calculation methods for potentiometric titration curves

| | | | |
|---|---|---|---|
| [20] | General | | End-point of potentiomet. titn. at the steepest inflection of titn. curve |
| [21] | Redox, pH | | Linearization of titn. curve. The most widely used method for detn. of end-point |
| [22] | General | | Graphical determination of end-point by the "circle method" |
| [23] | redox | | Exact numerical calculation of titn. curves with four equations. Definition of inhomogeneous redox systems |
| [24] | General | | Rigorous least-squares adjustment for calculation of non-linear equations |
| [25] | pH, redox | ALGOL | HALTAFALL program |
| [26] | pH, redox | ALGOL | Computer calcn. of titn. curves in multi-component systems |
| [27] | pH, redox | | Calcn. of redox titn. curves. Proved: equivalence point = inflection point |
| [28] | pH | ALGOL | Improved linear titn. plots, with activity coefficients |
| [29] | General | | Numerical methods for data-fitting problems. Detailed review and comparison of methods |
| [30] | pH | | Learning machine method for calcn. of titn. curves by multiparametric curve-fitting |
| [31] | | FORTRAN | Non-linear least-squares approach. Simplified LETAGROP=ACBA. |
| [32] | pH | | Calculation of pH titn. curves and end-points. Iterative method with interval halving |
| [33] | pH | | Unified calcn. of titn. curves (for limited number of components) |
| [34] | General | | Multi-component analysis computations using Kalman filtering |
| [35] | pH, redox | | Titration assisted by microcomputers. Electro-activity treated similarly to pH |
| [36] | pH | | Approximation equation for mixtures of acids and bases. Explicit equation for $[H^+]$ in simple cases |
| [37] | General | FORTRAN | Resolution of overlapping electrochem. peaks with Kalman filtering |
| [38] | pH | BASIC | TITFIT, a comprehensive program, Newton-Gauss-Marquardt method |
| [39] | pH | | Calcn. using $[H^+]$ as independent and $[B^+]$ as dependent variable using pocket calculators |
| [40] | pH | BASIC | Desk-computer program MINIPOT; use of Gauss and Wentworth method |
| [41] | pH | | The limit of separation of two weak acids |
| [42] | pH, redox | | Titration in a mixture with resolution of difference UV-visible spectra |
| [43] | pH | FORTRAN | Data analysis for up to nine components with TITAN program |
| [44] | pH | | Bjerrum plots for determinationn of systematic conc. errors |
| [45] | pH, redox | | Evaluation of digital potentiometric titns. by the Tubbs method |

Experience has shown that bi-weekly checks in these systems are warranted and that the corrective action should be taken at that time if necessary. The check should usually include checking the sample system, pressures, temperatures and reagent levels, ensuring that the reaction cell is operating as expected, and checking that no error messages are displayed on the digital panel meter. These messages are rarely transmitted to any receivers, panel instruments or other data terminals; in fact, when the instrument diagnoses a problem, it displays an error message on its digital panel meter and holds its last good analogue ouput so that process control upsets are minimized or avoided.

## 13.2 ESSENTIAL COMPONENTS OF AN AUTOMATIC TITRATOR

A titration system usually consists of the elements depicted in Fig. 13.1, namely (a) titrant delivery system, (b) titration vessel, (c) titration monitor and (d) control section.
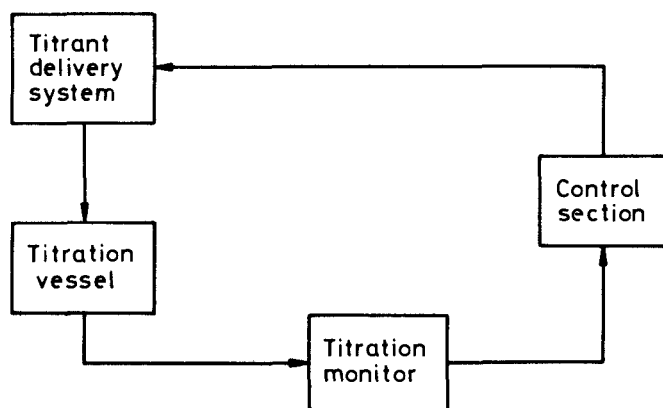


**Fig. 13.1**  Components of an automatic titrator.

### 13.2.1 Titrant delivery system

The development of automatic reagent delivery systems and their use in titrimetric analysis was a major step in automating titrimetric methods. One of the most difficult tasks involved in constructing an automatic titrator is probably the design of a sufficiently precise mechanism for reagent delivery. Currently, whole ranges of digitally controlled and programmable, high-precision dispensers are marketed, both separately and as part of commercially available titrators.

Reagent dispensers are chiefly volumetric or coulometric in nature. Former-ly, the automation of coulometric titration procedures developed at a higher speed than that of volumetric titration —this was probably a result of the ease with which the current intensity used for reagent generation could be reg-ulated by means of relatively simple electronics.

The commonest automatic titrators used in the laboratory are burettes, which can be classified according to their operational foundation into (a) burettes with automatic valves, (b) automatic syringe burettes and (c) other types of automatic burette.

(a) The typical burettes  with automatic stopcocks resemble  those used in classical titrimetric analysis —a longish, thin glass tube with a volume scale, equipped with a valve that can be operated automatically. Occasionally, there are facilities for automatic refilling and zero setting. Of the variety of automatic burettes described in the literature, those with mechanical [47] and electromagnetic [48] stopcocks, electromagnetic stopper valves [49,50] and electromagnetic pincer valves are the most widely used —particularly the last, commercially available from Radiometer, Beckman and a few other firms.

(b) Modern automatic titrators usually include syringe (piston or plunger) burettes from which the titrant can be dispensed at a preset rate. The burette resembles a medical syringe. The tube is made of glass or a rigid plastic ma-terial to a precisely uniform diameter. The plunger, made of glass, corrosion-resistant metal or, preferably PTFE, is also manufactured to a high precision so as to move freely inside the tube and yeat provide a leak-proof seal. One of the ends of the tube is fitted with a three-way glass stopcock and the other is used to dispense the reagent. One outlet of the burette is thus con-nected to the titrant reservoir and the other leads into the solution to be titrated. Both ends can be fitted with plastic or glass tubes. The burette tip should be sufficiently thin (e.g. a capillary) and conveniently reach into the sample solution. Most often, the burette is mounted upright, with the end pointing upwards. The dipensed volume is measured from the plunger displace-ment and is usually displayed on a digital counter. The electrical signal for switching the burette on and off is imposed on a small electric motor.

(c) Other types of automatic burette are also occasionally used. Thus, constant-flow burettes [51] overcome the problem of non-uniform rate of deliv-ery arising from the use of ordinary burettes by keeping the hydrostatic press-ure constant. The amount of titrant dispensed is proportional to time, so that the burette can be coupled to a t-y recorder to obtain a calibration graph. The time axis of the chart can be scaled in volume units. The relatively sim-ple recording burette [52] is capable  of recording the volume (height of the meniscus) by means of flexible metal bellows accommodated in the burette tube.

Hieftje and Mandarano [53] developed an ingenious system in which the ti-trant is delivered as uniform submicrolitre droplets at a rate controlled by an electronic digital pulse train. The device, shown in Fig. 13.2, is based on a principle first described by Lord Rayleigh [54] and later implemented by Lindblad and Schneider [55]. With this device, the titrant solution is fully converted to uniformly sized and spaced droplets, any of which can be sent into the titration vessel. To accomplish this, a liquid jet is formed by forc-ing a constant flow of titrant through a hypodermic needle. Ordinarily, a liquid jet formed in this manner would disintegrate into randomly sized and spaced droplets, in accordance with the natural disturbances within the jet. However, by artificially establishing a sufficiently intense periodic disturb-ance on the jet, it can be forced to break up into uniform droplets. In this system, the artificial disturbance is launched on to the jet by vibrating the needle in a direction normal to the jet. This is accomplished by attaching the needle to a piezoelectric bimorph strip which is driven by a variable-fre-quency power oscillator. In order to control the number of droplets, these must be extracted from the primary stream issued from the hypodermic needle, which is effected by passing the stream of droplets through a circular charg-
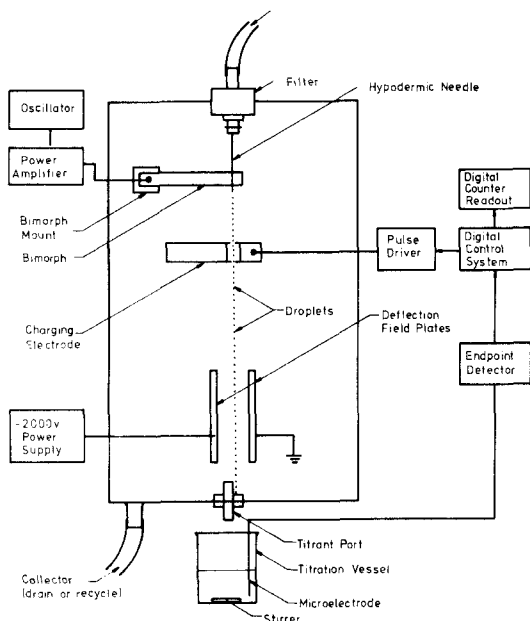


**Fig. 13.2** Scheme of the titrant delivery unit devised by Hieftje and Mandarano showing the control and measuring systems. (Reproduced from [53] with permission of the American Chemical Society).

ing electrode placed at a point where the droplets break off from the jet. An adequate voltage applied to the charging electrode then repels charges back into the jet so that droplets breaking off from the jet at that time retain a deficiency of the charge. In this manner, a single droplet or a large number of them can be charged by simply applying either a pulse or a constant voltage to the charging electrode. For digital control, one voltage pulse is applied to the electrode for each droplet to be charged. The charged droplets are then deflected by passing the entire droplet stream through a high-voltage d.c. field. Deflected droplets pass through the titrant port while the uncharged droplets are trapped at the bottom of the titrator and drain from the enclosure to be recycled or discarded. As the titrant is delivered as uniform droplets, the volume of titrant added is proportional to the total number of drops deflected, which is in turn equal to the number of pulses applied to the charging electrode. Consequently, the titrant volume can be directly read from a digital counter connected to the digital control system output or determined by digitally measuring the frequency at which pulses are applied. Alternatively, the digital output can be directed to a digital computer for computational convenience or control purposes.

### 13.2.2 Titration vessel

The characteristics of the vessel where the sample and titrant meet depend to a great extent on those of monitoring system used. Thus, while electroanalytical or photometric probes do not require special vessels (a simple beaker, thermostated if the chemical system involves demands it, where the probe can be conveniently submerged), conventional optical (photometric or fluorimetric) detectors require titration units matched to the design of the measuring compartment of the instrument, and thermometric detectors require not only the titration cell but also its environment to be thermally isolated.

### 13.2.3 Titration monitor

The most convenient systems used to monitor titrations are either electroanalytical or optical probes. In this respect it is worth emphasizing the greater development of potentiometric titrations, due to their low running and maintenance costs, and to the advent of ion-selective electrodes, which have enormously expanded the scope of application of potentiometric measurements.

On the other hand, amperometric measurements are confronted with the risk of electrode poisoning during the relatively long periods of time over which the electrodes are in contact with the unknown solution. Such a risk is even greater with organic compounds and precipitation titrimetry.

The use of optical fibre in titration probes has allowed the development

of commercial photometric titrators with brilliant performances, as will be seen later.

Other sensing systems, including fluorimetric, photometric and thermometric detectors, have been less frequently used in this area of laboratory automation.

### 13.2.4 Control section

This is the unit that actually reflects the degree of automation of the instrument and is thus dealt with in greater detail in Section 13.3.

## 13.3 DEGREES OF AUTOMATION

According to the classification established by Pungor *et al.* [56], there are three levels of automatic titrators defining as many degrees of automation, namely (a) hardware-controlled titrators, (b) microprocessor-controlled titrators and (c) computer-controlled titrators.

### 13.3.1 Hardware-controlled titrators

In automatic titrators, reagent dispensing is frequently controlled in a simple manner by use of detector signal feedback. One of the simplest and commonest methods of hastening the titration is the anticipation principle, based on a preset anticipation detector signal level. The reagent is added at a relatively rapid rate until the measured signal attains the preset level, from which the rate is decreased. In this way, the titration rate is increased with no significant detriment to the precision.

This operating mode is used in, among others, the Metrohm E-536 potentiograph, depicted in Fig. 13.3. It is a recording instrument for plotting titration curves of the most varied types. When connected to a Titrating Stand (a Dosimat pint buretter with swing-out magnetic stirrer), curves of the type electrode-assembly voltage *vs.* reagent volume (or their first differentials) can be recorded. The feed rate (burette piston speed) may be either constant or dependent on the slope of the titration curve.

The instrument thus covers the whole range of potentiometric methods, such as acidimetric (pH), ion-specific (pIon), redox, precipitation, complexometric and non-aqueous titrations. The use of pre-input converting units, which transform measured values into d.c. signals, allows the application to be extended so as to cover methods using polarized electrodes (amperometry and voltammetry), conductimetry and colorimetry. If needed, a Coulostat with an integrator unit may replace the burette for the coulometric generation of reagent.

The E536 differs from earlier Metrohm instruments in that the rigid mechan-

ical coupling between the Potentiograph and the Dosimat has been replaced with an electrical connection, and that differentiation is no longer a time function (dU/dt), but is based directly on the reagent volume (dU/dV), while the operation has been simplified by the provision of extra paddle switches for the realization of routine operations. The criteria used for operation with automated systems are fed out of socket connections, so that the Potentiograph may be connected directly to an automatic analyser with a sample changer (e.g. the E 552/12).



**Fig. 13.3** Hardware-controlled Metrohm E536 titrator. (Reproduced with permission of Metrohm Herisau, AG).

The Radiometer RTS 822 titrator is similar in performance to the Metrohm titrator described above.

Figure 13.4 shows the hardware digital logic circuit acting as a timing and decision unit in the titrator designed by Hieftje *et al.* [57], the titrant dispensing system of which was described above [53]. The circuit operates as follows: the input signal from the titration monitor is examined by analogue comparators $C_1$ and $C_2$, both of which are initially in a logical zero state. Provided the input voltage is less than the reference level of $C_1$, the output from $C_1$ stays at logical zero, so that gates $G_2$ and $G_3$ are open, and all the

pulses from the oscillator reach the timing unit. When the voltage from the titration monitor attains the reference level of $C_1$, the comparator changes states, closing $G_2$ and opening $G_1$, so that every $10^n$-th pulse reaches the timing unit. A rate reduction by a factor of 10 or 100 is thus readily switch-selectable.
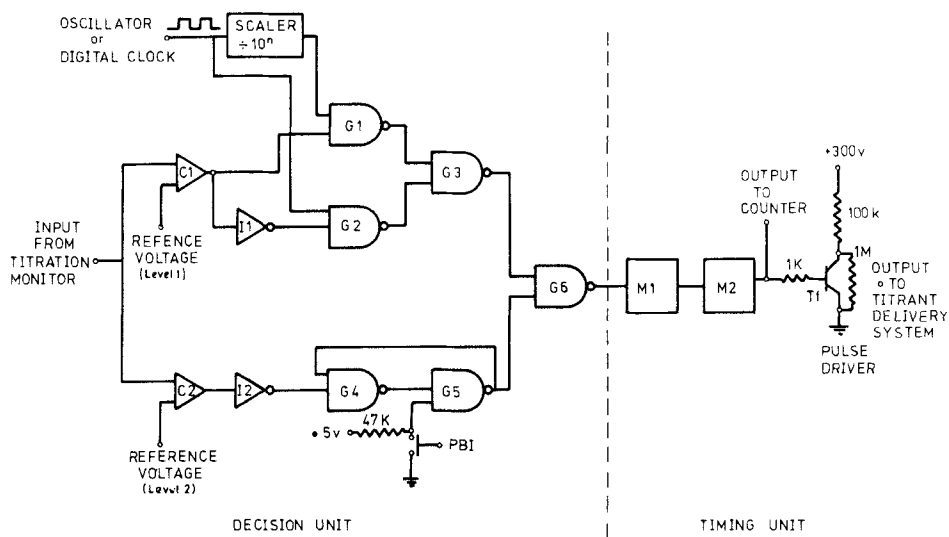


**Fig. 13.4** Scheme of logic circuit of the hardware-controlled titrator designed by Hieftje *et al.* $C_1$ and $C_2$ are comparators; $G_1$-$G_6$ are gates; $I_1$ and $I_2$ are inverters; $M_1$ and $M_2$ denote monostables adjustable from 100 μs to 1 ms and $T_1$ denotes a 2N3439 transistor. (Reproduced from [57] with permission of the American Chemical Society).

When the output from the titration monitor reaches the reference level of comparator $G_2$, the comparator output goes to a 1 state and resets to zero the output from the R-S flip-flop formed by gates $G_4$ and $G_5$. This closes $G_6$ and halts the titration. To initiate a new one, push-button $PB_1$ is pressed to set to a logical state of 1 the flip-flop formed by $G_4$ and $G_5$ and open gate $G_6$. The time unit used consists of monostables $M_1$ and $M_2$, plus the pulse driver. Monostable $M_1$ determines the delay time between the generation of a pulse by the decision unit and its appearance at the titrator, while $M_2$ determines the width of the pulse sent to the titrator. These parameters are adjusted by potentiometers for proper operation of the titrant delivery system and need be

set only once for each titrant during the initial set-up. The output from the monostable network is sent to the pulse driver, used to convert pulses from $M_2$ to an amplitude of about 300 V, suitable for controlling the titrant delivery and to a digital counter.

The determination of water by the Karl Fischer method is one of the commonest electrochemical titrations, and there are a variety of commercial instruments available for its routine implementation. Ziegler *et al.* [58] developed straightforward automatic amperometric and potentiometric titrators to perform a critical evaluation of the Karl Fischer method (Fig. 13.5). The amperometric circuit (Fig. 13.5a) includes a dummy cell or standard resistance to facilitate the adjustment (or calibration) of the applied potential to 200 mV without any auxiliary current or voltage-measuring apparatus. This elementary amperometric KF titrator was further developed to automatically control either the Sargent Constant Rate Buret or an automatic burette with a solenoid valve. An Assembly Products (API) 200-μA FS meter relay with 'lo' and 'hi' adjustable set points replaced the regular meter movement shown in Fig. 13.5b. The precision of the automatic titrator was evaluated by determining the volumetric ratio of a KF reagent *vs.* a water standard in methanol solution at separate intervals over a period of several days. On comparing the results obtained in replicate direct titrations with several concentrations of stabilized KF reagent, the precision achieved with the titrator was found to equal or exceed the reading error of the burettes. The elementary constant-current potentiometric apparatus used by Ziegler *et al.* is depicted in Fig. 13.5c. The potentiometric titration curves run for water in methanol with the KF reagent in the vicinity of the end-point at a constant current of 10 and 100 μA show that the instrument is about 10 times more sensitive than that described in the ASTM procedure.

The automation of thermometric or catalytic thermometric titrations began with the continuous addition of titrant from a flow burette and later with the use of the syringe-burette, and continued with the introduction of electrical temperature detection and recording of the titration curve. Full automation [59] was achieved by using a digital timer and constant rate of titrant addition, together with the use of a transistor circuit to convert the temperature change into a linearly decreasing voltage which was differentiated to give a square-wave voltage of amplitude proportional to the rate of temperature change and period equal to the duration of the change. The signal was amplified and fed to the end-point detector. Stern *et al.* [60] amplified the out-of-balance potential from the Wheatstone bridge. The potential was then filtered, differentiated and applied to the control unit of a Sargent-Malmstadt automatic titrator which amplified the signal and differentiated it twice; the

a)
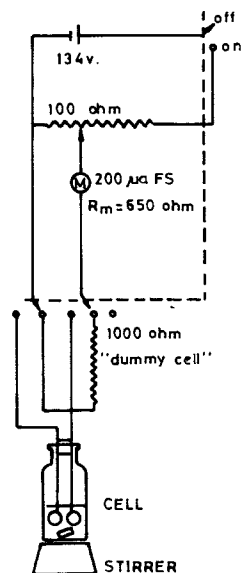
b)
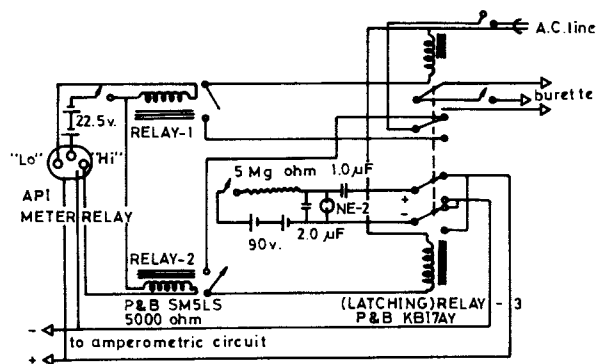
c)
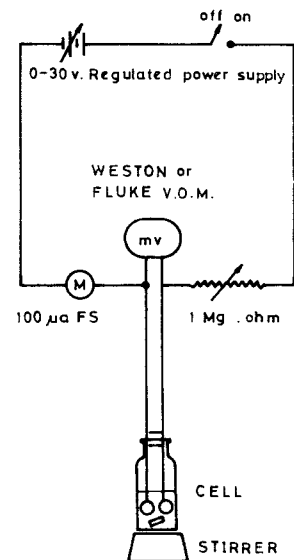


**Fig. 13.5** Instrumentation for critical evaluation of the Karl Fischer water method. (a) Amperometric circuit; (b) relay circuit for automatic burette control; (c) potentiometric circuit. (Reproduced from [58] with permission of the American Chemical Society).

abrupt signal change at the end-point then triggered a relay system to stop the titration.

### 13.3.2 Microprocessor-controlled titrators

There is now a wide range of commercially available, highly automated digital titrators accommodating built-in microprocessors, a genuine representative of which is the Memotitrator DL40 manufactured by Mettler. This is an independently operating, microprocessor-controlled compact instrument for individual titrimetric analyses. It is furnished with storage facilities for storing the titrations methods more frequently employed by the user, plus other information. The microprocessor is the heart of the instrument, depicted in Fig. 13.6. All operations related to data entry, sequency control, calculation, data output and so forth are handled by this unit. The programs for titration control, evaluation methods, standard parameter sets and multilingual texts are stored in the program-and-data storage. Data generated during the instrument's operation and information entered by the user are also stored. The keyboard is used to enter operating commands and other information. Depending on the prevailing conditions, the instrument determines which keys can be operated and in what sequence. This microprocessor-controlled activation of
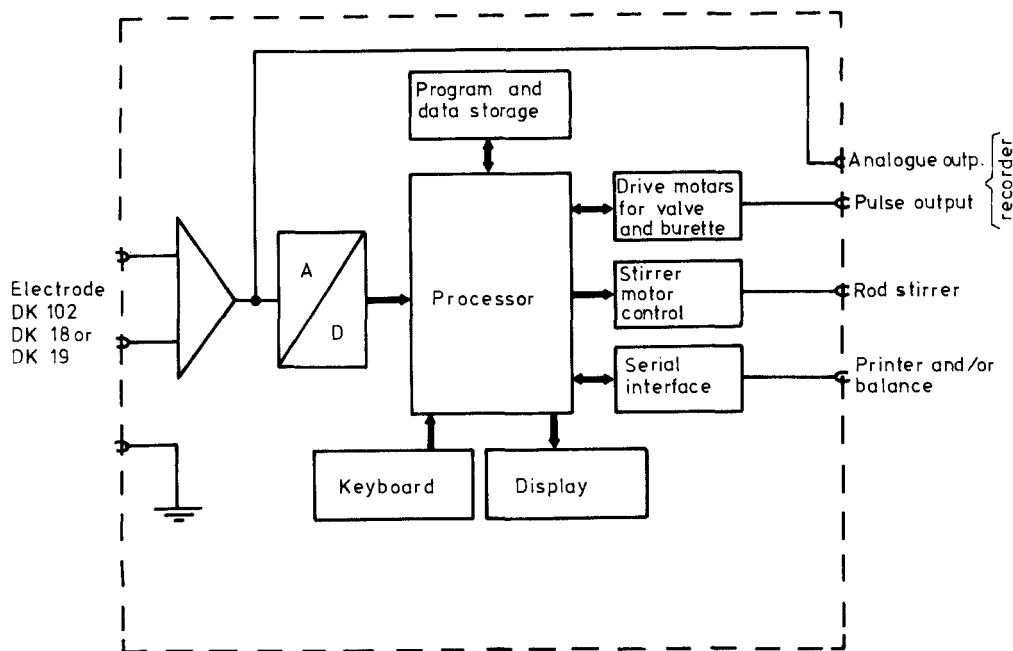


**Fig. 13.6** *Simplified scheme of the Memotitrator DL40. (Courtesy of Mettler Instruments AG).*

the function keys prevents operating errors. Results, input and output data and various messages are shown on a 20-place display.

The Memotitrator DL40 is capable of independently performing the following functions: (a) implementing preliminary procedures; (c) controlling titration sequences; (c) driving interchangeable burettes; (d) evaluating measurement values; (e) guiding the user by a dialogue held via the keyboard and the alphanumeric display; (f) indicating entries and results in the desired units of measurement and generating messages; (g) storing methods, auxiliary information, measurement values and results; (h) recording titration results and other information by means of the Mettler GA40 alphanumeric printer; (i) automatic 'learning' of titration methods; (j) automatic calibration of (potentiometric) electrodes; (k) processing weighing (on-line) with the aid of a balance. In addition, the instrument is capable of implementing photometric and Karl-Fischer titrations by use of suitable peripherals and an attachable DK102 power supply.

Two phototitration modules, namely the Phototitrator DK18 and the Filter Phototitrator DK19, have been conceived to expand the scope of application of the Memotitrator DL40 by endowing it with the flexibility of photometric titrations in the visible range. The only difference between these two modules lies in the type of interference filter/monochromator used. The DK19 uses interchangeable plug-in filters, each of which has a specific wavelength, while the DK19 has a built-in scanning interference filter which uses its angle of rotation to modify the wavelength of the filtered light in a linear fashion, thus allowing additional absorption spectra to be measured. The phototitrator operates in the visible range (i.e. at wavelengths between 400 and 690 nm).

The Memotitrator can also be fitted with conventional optical detectors. Thus, a Perkin-Elmer 650-10S spectrofluorimeter has allowed the development of the direct catalytic titration of EDTA based on its inhibitory effect on the oxidation of 2-hydroxybenzaldehyde thiosemicarbazone by hydrogen peroxide, catalysed by iron(III), and the indirect determination of this and some alkaline-earth metal ions [61].

A special titration vessel with a side opening through which powdered solids are introduced with a weighing spoon is used for Karl Fischer titrations. These have a distinct feature in that the solvent must be titrated before the sample itself as it also contains water. To avoid dosing the solvent with every new sample, the vessel is furnished with a stopcock which allows draining of the solution to make room for the next sample and for the Karl Fischer reagent to be added.

Precipitation titrations usually benefit from the use of some protective dried gas ($CO_2$, $N_2$). The equalization of the pressure in the reservoir is ac-

complished via the mounted absorption tube. The interchangeable burette is furnished with a tube to protect the reagent from light.

Voltammetric end-point detection with the Memotitrator calls for the use of a dual platinum pin electrode and a polarized power supply.

Metrohm markets microprocessor-controlled instruments for routine coulo-metric (KF 652 Processor) and amperometric-voltammetric (EP/KF 678 Processor) analyses. These instruments are frequently used for research purposes. Thus, the Mettler Memotitrator and the Metrohm Titroprocessor 636/Rod Stirrer 622 were used by Johansson *et al.* [62] to demonstrate that potentiometric two-phase titrations can be carried out in an automatic fashion. The typical background noise from the electrodes can be reduced by introducing hydrophobic anions or cations in the aqueous phase. These ions also affect the acid-base equilibrium by extracting the sample ions as ion-pairs into the organic phase, which allows the conditional acidity constant (apparent $K_a$) to be manipulated to make selective titrations possible.

Betteridge *et al.* [63] designed an automatic, microprocessor-controlled titrator for the determination of adipic and boric acid used in nylon manu-facture. The instrument, depicted in Fig. 13.7, is capable of recording the entire titration curve. During the titration, the titrator adds reagent por-tions of a preset volume and in a preselected number to the cell. Reagent addition, effected by a Radiometer ABU 13 autoburette, is controlled by the microprocessor via a read delay switch as requested by the program. The inter-val between individual reagent additions is kept constant throughout the titra-tion. Once a preset time has elapsed from the reagent addition, the cell po-
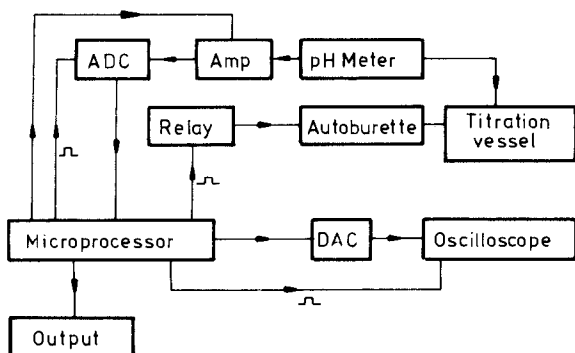


**Fig. 13.7** Scheme of the microprocessor-controlled titrator developed by Betteridge *et al.* (Reproduced from [63] with permission of the Royal Society of Chemistry).

tential values are entered in the microcomputer's memory after passing
through the pH-meter, amplifier and AD converter.

The titration end-point can be determined in a variety of ways. Betteridge
*et al.*   found that neither the Gran transformation nor the application of
similar functions provided sufficiently accurate results. The best results
were in fact obtained by plotting the differences of the neighbouring elec-
trode potential values as a function of the reagent volume added and by search-
ing for its maximum. The obtainment of accurate results required curve smooth-
ing. The authors tried three different smoothing techniques (running average
of three or five, median smoothing and Hanning smoothing) and found the differ-
ential titration curve obtained by running an average of three to be   the
least noisy.

The hardware used consists of an AD converter connected to an amplifier, a
relay switch (operated by the microprocessor) for titrant portioning and a DAC
unit for decoding of data obtained and stored by the microprocessor. The micro-
computer assembled by Betteridge *et al.* for this purpose consisted of 1kb ROM,
2kb RAM and a digital I/O unit. The main functions of the software are data
collection and display, derivation, curve smoothing, peak detection, interpola-
tion (determination of the maximum slope of the titration curve by calcula-
tion) and result delivery (display).

By carrying out titrations under different experimental conditions, the
authors chose the most suitable conditions for measurement (ADC range, stir-
ring rate, time interval between titrant additions and digitizing voltage).
Under optimum conditions, they could determine the concentration of adipic
acid with an error of about $\pm 0.2\%$ and that of boric acid with an error of
$\pm 0.1\%$ —the latter without the addition of mannite. The time required for each
analysis was between 1 and 15 min, depending on the time needed to reach a
constant potential in an interval.

### 13.3.3 Computer-controlled titrators

Titration instruments fully controlled by a computer through appropriate
software represent the highest degree of automation available in titrimetric
analysis today.

A computer can control a variety of functions performed by titrators, name-
ly: (a) observation of the change in the detector signal level and acquisition
of information on reaching the steady-state within a given precision; (b) cal-
culation of the amount of reagent to be added in the next step; (c) commanding
the addition of the next amount of reagent or refill the burette; (d) storing
and monitoring of the amount of reagent added (detector signal data pairs);
(c) searching for the equivalence point and end-point.

In 1975, Hieftje *et al.* [57] evaluated several configurations differing in the method used to control titrant delivery (either computer, hardware, or manually controlled) and to detect the end-point (either fixed-level or derivative). The system for titrant delivery and the hardware were described above in Sections 13.2.1 and 13.2.4, respectively. The computer-controlled titrator uses the electrode-electrometer combination described above as a titration monitor. However, the decision and timing units and the counter are all embodied in the computer software, a block diagram of which is shown in Fig. 13.8. To start the program, the operator supplies the computer with a set of initialization parameters including the initial and reduced rate of titrant addition, the type of data pretreatment to be performed, the location of mass storage where the data are to be stored and the end-point detection method to be used. Once the program has been initiated, the titration is started upon operator command; after this command, and at the time of each synchronization pulse from the titrant delivery system, the decision unit determines whether to add a droplet of titrant, based on the information from the titration monitor. If a droplet is to be added, this decision is passed on to the timing unit, which generates a pulse of the delay and length required by the titrant delivery system: each time a droplet of titrant is added to the titration vessel, the voltage from the titration monitor is digitized and stored as a data point. After treatment, the data are sent to the decision unit and displayed on a cathode ray tube. The titration curve is thus displayed in real time during the titration, so that at any time the operator can override the decision unit. This permits manual intervention in the case of non-routine ti-
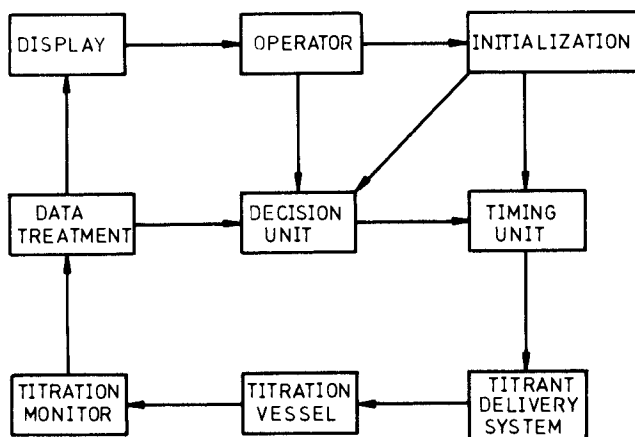


**Fig. 13.8** Operational scheme of the computer-controlled titrator developed by Hieftje *et al.* (Reproduced from [57] with permission of the American Chemical Society).

trations. Decisions concerning the rate of titration and end-point location may be based on the input data or on their first or second derivative. Thus, three software end-point detection techniques are available for use with the computerized titrator. A fixed-level technique, similar to that used by hardware-controlled titrators, employs reference levels which are numbers stored in the computer, thereby overcoming the need for highly stable voltage sources.

The computerized titrator offers several advantages over the harware system. Some of these advantages are of particular importance when derivative end-point detection is applied, which avoids drifts in the reference levels and the titration monitor and the need to know the absolute titration monitor voltage at the equivalence point. The system can be used with first and second-derivative end-point detection and is relatively immune to noise thanks to the computer's ability to smooth incoming data digitally. Another advantage of the computer is its ability to store the titration data on magnetic tape, thereby allowing more sophisticated data processing techniques (e.g Gran plots [64] and interactive data analysis [65]) to be performed after the titration is complete. The best performance (0.16% relative standard deviation) is obtained with this computer-controlled configuration by using the first or second-derivative end-point method. The computer-controlled titrator is also superior *in* versatility, in the choice of end-point detection and in flexibility for adaptation to non-routine analyses using operating interaction.

In all the titrator configurations examined by Hieftje *et al.*, the precision-limiting factor seemed to be the end-point detection process rather than the titrant delivery system.

Improvement of precision has been the target of much research involving the different types of sensing and chemical systems typically used in titrations. Thus, Frazer *et al.* [66] use a system consisting of ion-selective electrodes (ISEs), an automatic burette, interface hardware, a minicomputer, a cathode ray tube (CRT) display, a light pen, a teletypewriter and a special function panel (Fig. 13.9). Each of the system components performs the task for which it is best suited and provides for scientist-computer interactions. The system software changes often required for the development of new methods and semi-routine determinations are written in a high-level language, FOCAL. The system delivers the titration curve, the equivalence points of which are determined by least-squares regression extrapolation, via the CRT display and allows Gran plots, second derivatives and error functions to be obtained, and also  as well as the implementation of interactive data-reduction techniques. This original configuration was later improved by its proponents for the determination of end-points in potentiometric titrations. The titration rate and

data acquisition are under the computer's control. The potentiometric curve and Gran (antilog) plot are displayed on the CRT as the titration proceeds. Once the titration has been completed, the operator can use interactive graphics techniques to query the Gran plot and develop an error function which indentifies the period during the titration when the cell output is most nearly Nernstian. The data obtained during the identified period are used to estimate the equivalence point [67].
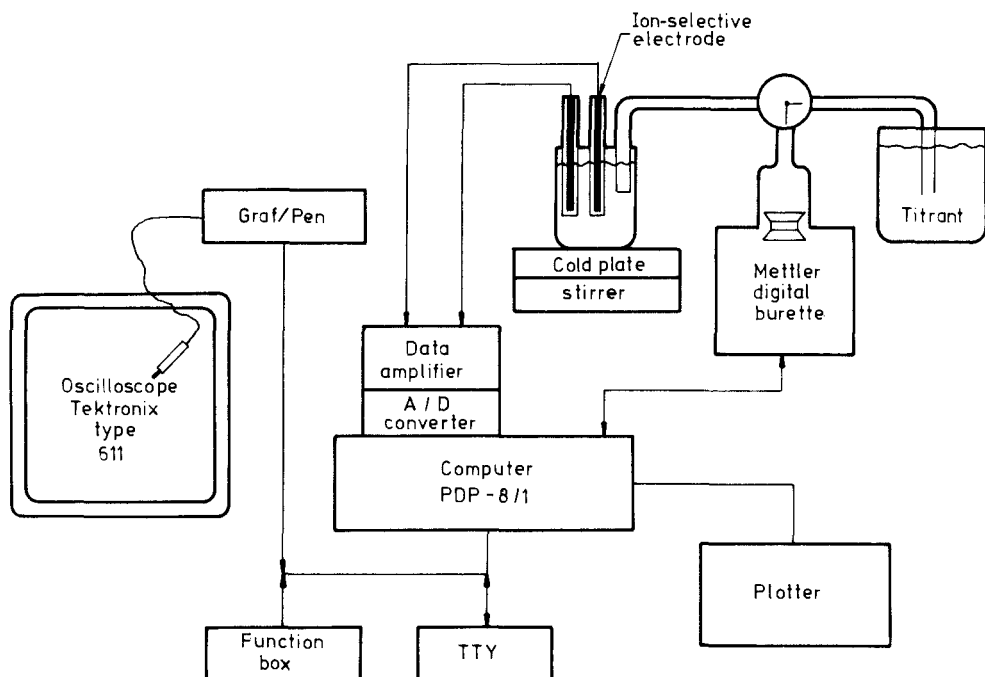


**Fig. 13.9** Computer-controlled titration system. (Reproduced from [65] with permission of the American Chemical Society).

Martin and Freiser [14] designed a microcomputer-controlled potentiometric system combining the low cost and ease of construction of microcomputer-based instrumentation and the high potential of minicomputer software. A scheme of the computer and associated hardware is shown in Fig. 13.10. All components except the electrode multiplexer are commercially available. The computer is an IMSAI 8080 kit equipped with 24K of static memory. All interfacing is effected through Processor Technology, and the software is written in CONVERS [68], a language based on an interpretative compiler and combining the conversational and ready debugging of an interpreter-type language with the typical speed of a compiler. To demonstrate the system capabilities, its propo-

nents wrote software to locate end-points in a potentiometric titration and plot the titration curve, as well as to calibrate up to six ISEs and plot their calibration graphs. The potentiometric titration program uses routines similar to that described by Christiansen *et al.* [4] to calculate the desired titrant increment volume (DTIV) and locate end-points. The DTIV is calculated on the basis of the magnitude of the first derivative of the titration curve, while the end-point is located by calculating the second derivative and extrapolating between the last positive and first negative values. Two routines are used; one for acid-base titrations in which the meter is used in the pH mode and the other for general potentiometric titrations to determine which mode is operative and implement the appropriate routine. The system is capable of locating end-points in potentiometric titrations and generating calibration graphs data for multiple ISEs. It also allows the determination of $pK_a$'s and metal-ligand formation constants, the implementation of standard-addition and linear (Gran) titrations and the automatic determination of ISE selective coefficients.
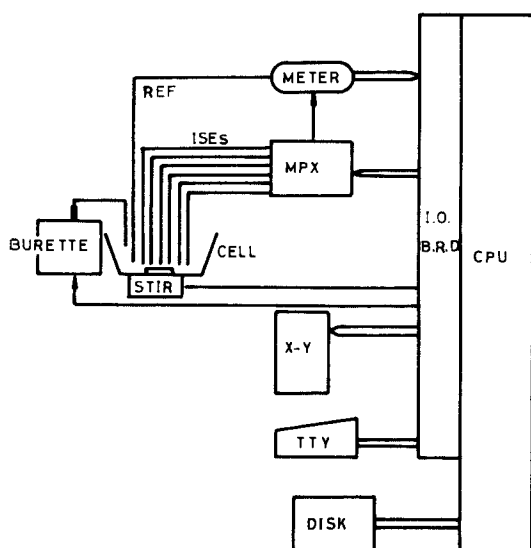


**Fig. 13.10** Diagram of the system developed by Martin and Freiser. (Reproduced from [14] with permission of the American Chemical Society).

Goode [69] developed computerized curve-fitting methods for the determination of end-points in spectrophotometric titrations. The titration curves are generated by numerical computation by considering the generic reaction

S + T ⇌ P

where S denotes the sample, T the titrant and P the product. The equilibrium of this reaction is described by the equation

$$K_{eq} = \frac{[P]}{[S][T]} \tag{1}$$

The titration curve is a plot of absorbance against the titrant volume. Light absorption is due exclusively to the product, so that

$$A = \epsilon b[P] \tag{2}$$

At any point along the titration curve,

$$[S] = C_S - [P] \tag{3}$$

$$[T] = C_T - [P] \tag{4}$$

where $C_S$ and $C_T$ are the analytical concentrations of the sample and titrant, respectively.

Equations (1)–(4) can be combined and solved for absorbance as follows:

$$A = \frac{\epsilon b\{\alpha+\beta+1-[(\alpha+\beta+1)^2 - 4\alpha\beta]^{1/2}\}}{2K_{eq}}$$

$$\alpha = \frac{K_{eq}V_S C_S}{V_S + V_T}$$

$$\beta = \frac{K_{eq}C_T V_T}{V_S + V_T}$$

where $V_S$ is the sample volume placed in the titration vessel and $V_T$ is the volume of titrant added at any point.

Titration curves are generated by computation of Eq. (5) by means of a FORTRAN program running on an IBM 370/168 computer. Computations are made under various conditions. Plots of all curves are carried out on a Calcomp flat-bed plotter interfaced to the computer. The titration curves can be ana-lysed by three different procedures. The equivalence point is determined by a derivative method, an extrapolation method and a non-linear least-squares curve-fitting method, the last being the best. The precision and accuracy achieved with the system vary according to the level of noise. If a titration with a large equilibrium constant and less than 0.004 units of noise is per-formed, the equivalence point can be determined to within a few tenths of a

percent and is generally limited by the precision to which the volume is measured. With small equilibrium constants, the curve-fitting method may be the only method capable of providing the location of the equivalence point. A titration curve obtained for an equilibrium constant of 100 will provide an answer accurate to within 5% provided that the noise is less than 0.01 absorbance unit. Neither the derivative method nor the extrapolation of linear segments provides acceptable results with such small equilibrium constants. In addition, the derivative method requires too many data points to be a good, general-purpose method for the location of the equivalence point. The nonlinear least-squares fit requires a large computer for implementation, although it calls for no on-line data acquisition. The titration curve run, usually consisting of 20 pairs of numbers, can be transmitted to a larger computer for analysis at a slightly later time. The data link can be high-speed dedicated lines if the titration data must be analysed within seconds, or punched cards if overnight computation is acceptable. One major application of this system is the analysis of titration curves obtained under adverse conditions such as low equilibrium constants, poor signal-to-noise ratios or low analyte concentrations. The non-linear least-squares fit again comes to the rescue when other techniques fail completely.

Campbell *et al.* [70] developed a BASIC program, LESTEQ, for calculation of the exact equivalence point volume in spectrophotometric titrations by use of ancillary indicators. The program is based on the non-linear least-squares fit of the titration data to a model equation by means of a modification of the Gauss–Newton multi-parameter optimization method, and calculates the indicator and stability constants. The form of the model equation used by LESTEQ depends on the stoichiometry of the titration reaction and on the nature of the indicator reaction. The equation

$$V_T = V_E - \frac{VK_T}{C_T} - \frac{VK_1(D_T - AV)}{C_T(AV - D_D)} + \frac{V_E K_T(AV - D_D)}{K_1(D_D - AV)}$$

is valid when the titrant and the titrand form a 1:1 complex and the indicator forms a 1:1 complex with the titrand before the equivalence point and exists in free form after it. This is the case with complexometric titrimetry using metallochromic indicators. The erroneous variable A (absorbance) is a function of (a) the error-free variable $V_T$ (volume of titrant added at any stage of the titration), (b) two known constants, $C_T$ (titrant concentration) and $V_I$ (initial volume of titrant) and (c) five unknown system parameters, namely $V_E$ (the equivalence point), $K_I$ and $k_T$ (the titrand–indicator and titrand–titrant conditional instability constants) and $D_D$ and $D_T$ (constants proportional to the

absorptivity of the indicator in its pre- and post-end-point forms, respectively).

The best possible estimates of the system parameters will correspond to the minimum sum of squares, $S_S = (A-A_C)^2$, where $A_C$ is the absorbance calculated from the model equation by using estimates of the system parameters. Hartley's modification [71] of the iterative Gauss–Newton [72] multi-parameter optimization method permits the values of the five system parameters yielding the minimum sum of squares to be found. The convergence of this method on the minimum sum of squares relies on a good initial approximation being input to the algorithm. Assuming —wrongly— A to be the error-free variable and $V_T$ to be the erroneous variable results in a linear model equation with two independent variables. By assuming $Y = V_T + (VK_T/C_T)$ to be the erroneous variable, and $X_1 = V(D_T-AV)/[(AV-D_D)C_T]$ and $X_2 = (AV-D_D)/(D_T-AV)$ to be the error-free variables, one may obtain estimates of $V_E$, $K_T$ and $K_I$ for known values of $D_D$ and $D_T$. In LESTEQ, the simplex search technique [73] allows the $D_D$ and $D_T$ values minimizing the sum of squares to be found and hence good initial estimates of the system parameters required for the Gauss–Newton algorithm to be obtained. The major advantage of the simplex search technique is that it converges towards the region of optimum response from a very poor initial approximation. LESTEQ provides more satisfactory results than the Keller–Richter method in terms of reliability and precision in estimating the equivalence point volume, by use of Mg–EDTA with Eriochrome Black T as indicator and an AMICA modular titrator, particularly with low concentrations. LESTEQ is also applicable to spectrophotometric titrations [74].

Efstathiou and Hadjiioannou [75] have investigated the problem of end-point detection in titrations based on slow reactions. They replaced the usual back-titration procedures by a new technique applicable as long as the reaction between titrand and titrant, however slow, is stoichiometric. This technique requires microcomputer control and allows automatic endpoint detection provided that certain preselected kinetic requirements are met. The technique is based on the following principle: compound C reacts slowly but stoichiometrically with compound T, with which it is to be titrated according to the reaction

$$C + T \xrightarrow{k} \text{products} \tag{I}$$

Assuming the availability of a transducer with a sufficiently fast response and capable of monitoring the concentration of T in the titrated solution, the following sequence of events, illustrated in Fig. 13.11, takes place:

(1) An initial volume, $V_W$, of solvent or buffer is transferred into the titration cell.

(2) A preselected volume of titrant, $V_0$, of concentration $[T]_0$ is automatically delivered to form a 'base' titrant of concentration $[T]_B$, given by $[T]_B = V_0[T]_0/(V_W+V_0)$.

(3) The corresponding 'base' signal of the transducer, $S_B$, is measured and stored in the computer memory.

(4) A sample volume, $V_S$, containing the whole amount of C to be titrated is transferred into the reaction cell to give an initial concentration $[C]_0$.

(5) The computer continuously receives the signal from the transducer, from which it calculates and stores the initial reaction rate, $R_0$ ($R_0=dS/dt$), in arbitrary units, proportional to $-d[T]/dt$: $-d[T]/dt = k[C]_0[T]_B$.

(6) The burette is then actuated and automatically delivers titrant until the signal from the transducer returns to the base signal $S_B$. Reaction (I) takes place during this addition, consuming T until the delivery rate exceeds the consumption rate and the concentration of T returns to $[T]_B$. The total volume of titrant delivered, $V_1$, is stored in memory. To avoid excessive overshoot of $S_B$, the delivery rate is made progressively smaller as the signal S approaches $S_B$.

(7) The computer, as in step (5), calculates the new reaction rate, $T_1$, which will be smaller than the initial rate $R_0$ insofar as part of the titrand will have been consumed.

Steps (6) and (7) are repeated with addition of volumes $V_2$, $V_3$, ..., $V_n$. The titration is finished when the reaction rate measured after the *n*-th delivery of titrant, $R_n$, is equal to or less than a preselected fraction of $R_0$ —typically $0.001R_0$, i.e. when 99.9% of the titrand originally present has reacted and the titration can be considered quantitative.

The corrected (equivalent) titrant volume is calculated by taking into account that the total titrant volume delivered for the titration of C, $V_F$, is equal to $V_1+V_2+...+V_n$. Volume $V_0$ is not included as it is delivered solely to form the base titrant concentration $[T]_B$, which is restored after the end of the titration sequence. The equivalence volume, $V_e$, must be calculated from $V_F$, allowing for the fact that dilution due to the sample volume and titrant additions requires the addition of further titrant in order to restore its concentration in the final solution to $[T]_B$.

Thus, at the end-point,

$$[T]_B = \frac{\text{Total equivalents of T added} - \text{Equivalents of T consumed by C}}{\text{Total volume in the titration cell}} =$$

$$= \frac{(V_0+V_F)[T]_0 - V_c[T]_0}{V_W+V_0+V_S+V_F}$$

which, combined with the equation in step (2), yields

$$V = \frac{V_F V_W - V_0 V_S}{V_W+V_0}$$
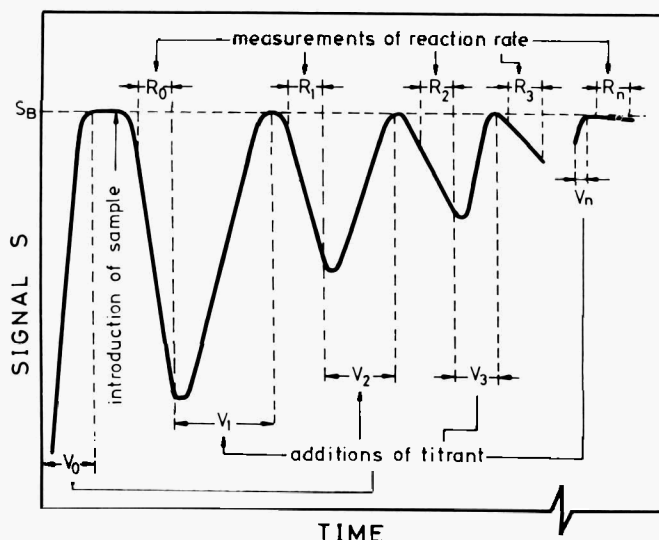


**Fig. 13.11**  Sequence of events  taking place during  a titration with kinetic  detection of the end-point. The scale of the time axis is not uniform along  its length. $v_0$, $v_1$,  ..., $v_n$  denote the  time periods during which  the titrant is delivered. $R_0$,  $R_1$, ..., $R_n$ represent the time periods  during which  reaction-rate measurements  take place. (Reproduced from [75] with permission of Pergamon Press Ltd).

The efficiency of this technique has been demonstrated by applying it to direct titrations of some polyhydroxy compounds with standard periodate solution. Thus, ethylene glycol and  propylene glycol (0.05–0.3 mmol), glycerol (0.06–0.17 mmol) and mannitol (0.01–0.03 mmol) can be determined with average relative errors of 0.1–0.3% by using a microcomputer-controlled potentiometric system consisting of the following commercially available units: an ALTAIR 8800B microcomputer equipped with a 32K RAM and the required peripherals (DRT,

teletypewriter, cassette recorder), an Orion 801 digital pH/mV–meter and a Sargent–Welch S–11120–12 multispeed burette driven by a stepper motor. The flow charts of the overall control program and two main subroutines employed are shown in Fig. 13.12.

OVERALL    PROGRAM                              SUBROUTINES
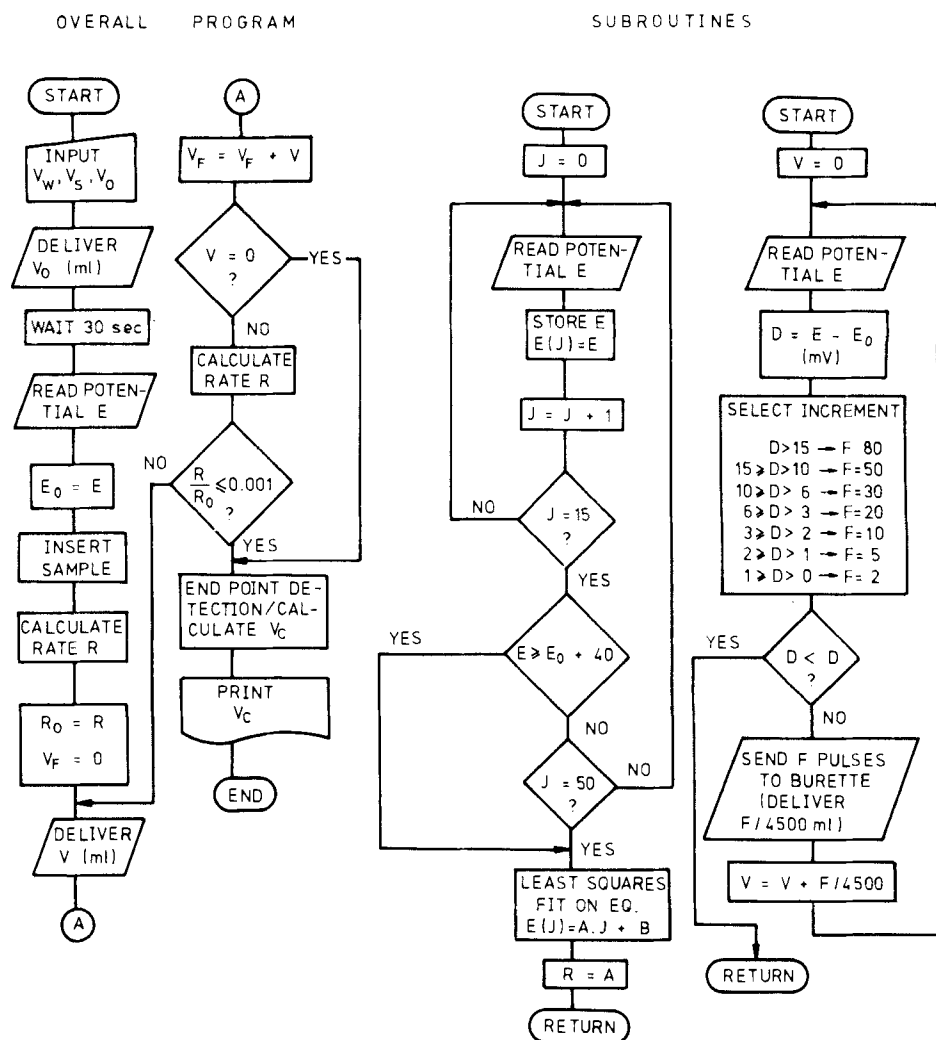


**Fig. 13.12** Flow charts of the overall control and two main subroutines for titration method with kinetic detection of the end-point. (Reproduced from [75] with permission of Pergamon Press Ltd).

Howard and Henzel [76] developed a coulometric titrator to control the titration rate in order to obtain constant changes in the pH with time (i.e. evenly spaced $\Delta$pH values), especially in the vicinity of the end-point to be determined. Although maintaining a constant rate of pH change prior to the end-point is time-consuming, the resulting values are particularly well suited to curve-fitting analyses. In order to be able to titrate relatively large samples (up to 100 $\mu$mol compared with the typical 0.1–10 $\mu$mol) in a reasonable time and still regain control without overshooting in the region of the equivalence point, they devised a control algorithm different from any reported previously [4,9,14], with the required dynamic range, rapid response and fine point control.

The coulometric titrator developed by Howard and Henzel consists of a titration cell, an electrochemical titrant generation system with a constant current source, an Orion 801A Ioanalyser pH-meter, a DEC PDP 8/E minicomputer, a teletype with a paper tape punch and the required interface hardware to allow the minicomputer to control the titrant generation rate, acquire the data and output them. The titration cell is a thermostated 30-mL weighing bottle fitted with a rubber stopper modified to accommodate the electrodes and allow for purging with nitrogen.

The software used allows the operator to enter the number of readings to be summed for each output, the number of readings to be delayed so that the solution may become more homogeneous, whether acid or base is to be generated, the desired pH change reading and the coefficients for the control algorithm. Most of the titrations carried out involved over 2000 data readings and typically 10–25 summed readings per datum output, with a pH change of 0.003 unit per reading. The closed loop control is based on a proportional plus integral plus differential plus squared (PIDS) algorithm that is used to calculate the time the titrant generation system should be on (TO) for each interval:

$$TO = LTO + (AE+BE[E]+C\Delta E[\Delta E])/(F\Delta pH/LTO) \tag{6}$$

where LTO is the previous TO, E is the error in the pH (E = measured pH – calculated desired pH for the present reading), $\Delta$pH is the change in the pH from the previous pH and A–F are constants entered during the initialization phase of the program and ranging between 4095 and its reciprocal. The term $\Delta$pH/LTO is used to allow for the non-linear variation of the pH as a function of the amount of titrant added. Both E, $\Delta$E and $\Delta$pH are multiplied by 1000 prior to introduction into Eq. (5). Small values are used for the coeficients of the squared terms so that they only become important when E or $\Delta$E is relatively large. A first-in, first-out (FIFO) rubber band storage routine is used to hold the data until they can be output to the teletype and paper tape punch.

The output routine for the pH and TO data pairs is a low-priority background program. The punched paper tape with the data is input into a DEC Edusystem 50 computer which is programmed in BASIC to analyse the data and display them on a Tektronix 4010 terminal. A typical titration requires about 30 min, and the analysis of data another 5.

Howard and Henzel applied their system to the titration of four samples of 35.82 $\mu$mol $Na_2CO_3$ and obtained a mean of 71.50 $\mu$mol of hydronium ion generated with a relative standard deviation +0.27%. The titrator was less than full on in the pH ranges 3.3–5.3 and 6.8–8.9. In these ranges, the measured pH was controlled to within 0.002 pH units of the desired value.

A compact automated titration system for the determination of calcium in sea water was designed by Anderson and Granéli and tried out successfully during the YMER-80 Arctic expedition [77]. The system burette (Fig. 13.13) acts both as a measuring device, a titration vessel and a cuvette. The burette is driven by a four-phase stepper motor and the up-and-down motion of the piston is determined by its direction of rotation. The unit is controlled by a Commodore PET 2001 computer equipped with 8kb RAM and 8kb ROM, and using cassette tape from program storage. It is usually programmed in BASIC, though
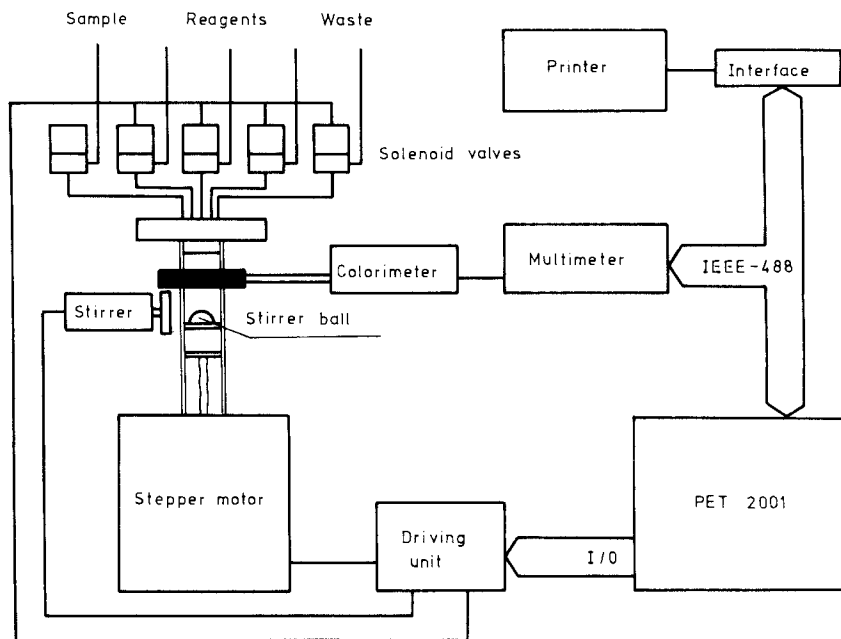
**Fig. 13.13** Schematic diagram of titration system for high-precision determination of calcium in sea water. (Reproduced from [77] with permission of Taylor & Francis Ltd).

it also accepts machine code subroutines. The titration system bus is connected to two devices: a printer, where results and primary data are reported, and a multimeter, which is connected to the colorimeter used. The parallel I/O port serves three functions, namely: (a) to drive the stepper motor (two lines); (b) to drive the stirrer (one line) and (3) to control the solenoid valves (three lines). One of the lines to the stepper motor controls the direction of rotation while the other is used to trigger the motor drive logic. The three lines to the valves are decoded so as to allow up to eight valves to be controlled.

This system was also used to implement the photometric titration of calcium in the presence of a higher concentration of magnesium reported by Jagner [78]. Calcium was titrated with EGTA at pH 8.6. A small amount of Zn–EGTA was also used and the decrease in the calcium concentration was followed by the simultaneous titration of zinc with Zincon as indicator. The experimental data for absorbance *vs.* titrant volume added (v) were linearized by the Gran function [64]

$$F = v_1(v_0+v)(A-A_{min})/(A_{max}-A)$$

where $v_0$ is the total volume (excluding that of the titrant), $A$, $A_{min}$ and $A_{max}$ are absorbance values (measured at 620 nm) and

$$v_1 = v + (v_0+v)[ZnOHI]/t_{EGTA}$$

or

$$v_1 = v + \frac{v_0[I]_{tot}}{t_{EGTA}(A-A_{min})/(A_{max}-A_{min})}$$

where $t_{EGTA}$ denoted the titrant concentration and $[I]_{tot}$ the total concentration of indicator at volume $v_0$. Volume $v_1$ is introduced to compensate for the EGTA released by the Zn–EGTA complex in the formation of the Zn–indicator complex.

Ten consecutive titrations of deep water (from 3000 m below sea level) carried out on board the YMER gave an average of 10.205 mMw (mmol/kg sea water). In practical work, the accuracy was set by the standardization of Na4EGTA. The time from the start of one titration to the beginning of the next was approximately 20 min.

Ionics Inc. are the designers and manufacturers of a multi-function digital analytical instrument suitable for making up to three simultaneous measurements on process solutions containing inorganics, reactive organics or dissolved metals [79]. Some of the measurements that can be made are acid–base

a: pH.
b: Selective ion electrode.
c: Colorimetric titration
   endpoint sensor.
d: Colorimeter coil.
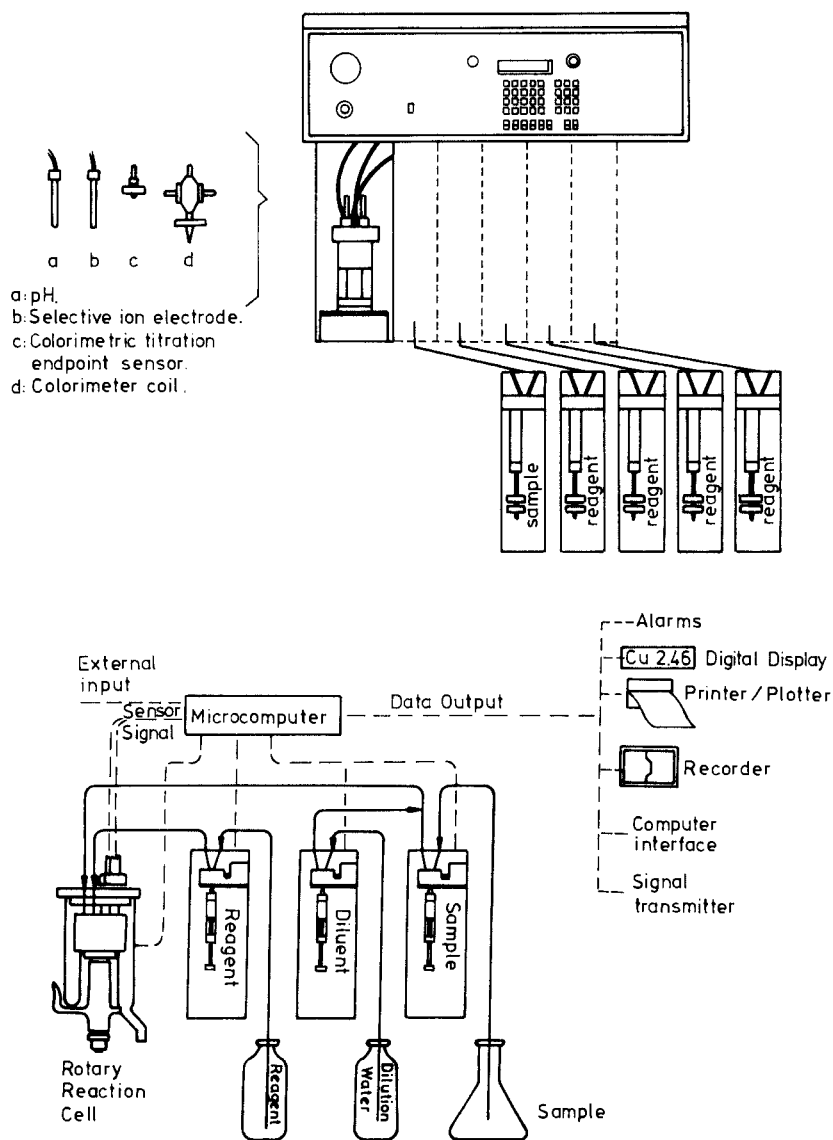
**Fig. 13.14** Scheme of automatic multi-function digital titrator.
(Courtesy of Ionic Inc.).

Karl Fischer or amperometric titrations; colour change end-points and colour absorption characteristics and specific ion concentrations. Functions are sequenced through a front panel keyboard or EPROM chip for sample fill, reagent delivery, spin, rinse , wait and detector sensing actions to enact the desired titrimetric procedure. Figure 13.14 shows four different sensors that can be used along the rotary reaction cell with up to five digitally controlled burette assemblies. Dilution water can be added to the reaction cell by means of a burette assembly or a fixed-volume solenoid valve. The basic operation of this instrument as an automatic titrator is as follows: the reaction cell is rotated slowly during most of the titration cycle; sample, diluent and various reagent burettes are sequentially filled with through air-operated, sliding ceramic valve assemblies. After the burettes have been filled, a small volume of liquid from each burette is dumped into the cell to ensure that any air bubbles present are expelled from the burettes; water is then added to the cell, which is rinsed and evacuated by using centrifugal force developed by spinning it at a high speed. A second water wash and rinse sequence is used to remove any trace impurities remaining in the cell after the first operation; a small preselected volume is then added with the reaction cell rotating slowly followed by a preset volume of diluent. After waiting for a few seconds to ensure complete mixing, the titrant is incrementally added in microlitre volumes until a specified end-point or pH is reached. When the titration is complete, the output is displayed on a digital panel meter and held until the next titration cycle. The panel meter, printer and/or recorder are updated at the end of each titration cycle. The wash, rinse and reagent addition cycle is then repeated as programmed via the keyboard RAM memory or permanent PROM memory.

Figure 13.15 shows the operational scheme of this automatic titrator. The heart of the unit is an INTEL 8080 microprocessor mounted on the central processing unit (CPU) board. The rotary reaction cell assembly can accommodate up to three different sensors for multiple measurements on the same processed sample. Each stepper burette board controls up to two burette dispensing assemblies. Function boards such as the colorimeter board, air burette board, E/I output board and RS-232 printer interface boards are available optionally. The optional D/A and E/I board is used for closed-loop applications where the titrator controls the final element such as a control valve. The RS-232 printer interface board is useful for troubleshooting the equipment and editing user-defined programs. The instrument accuracy, repeatability and response time vary widely and depend on the particular type of measurement concerned. The system requires a.c. power, a 75-psi air supply and a dilution water supply for proper operation. The air flow-rate required is of about 50 $cm^3$/min
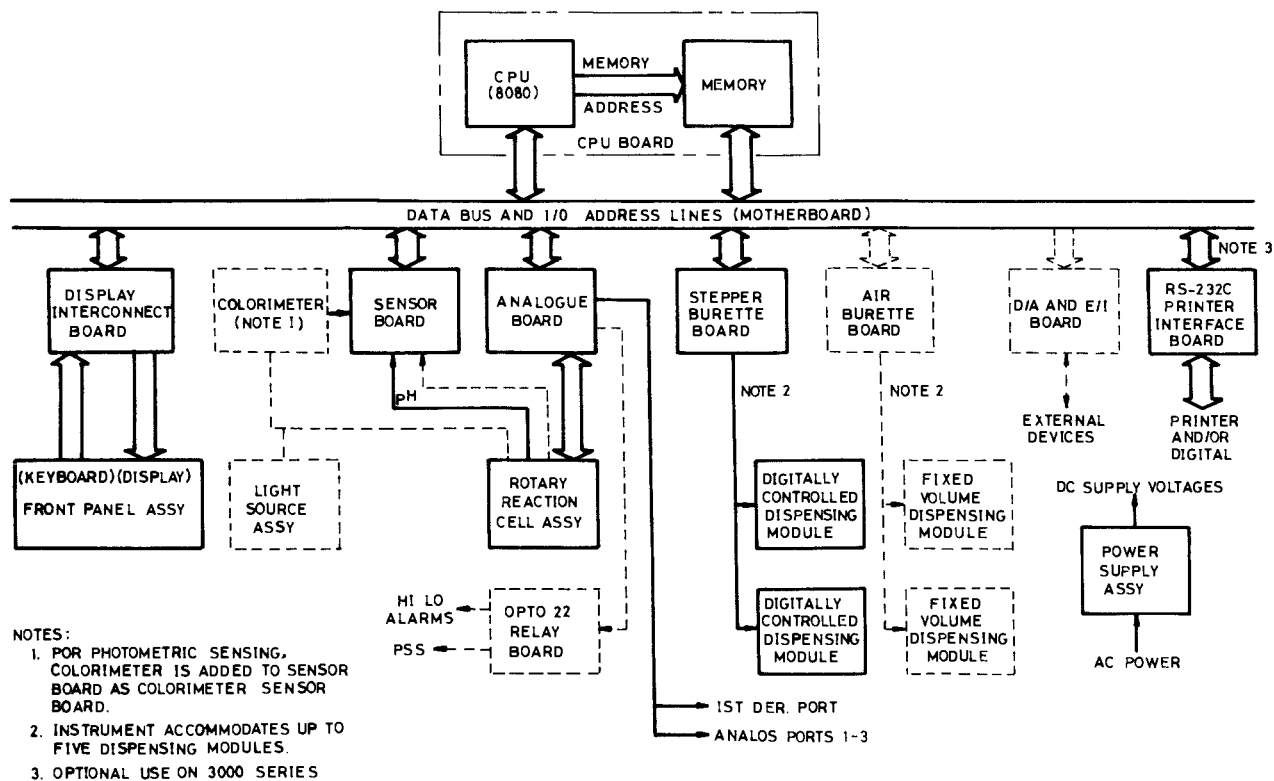
**Fig. 13.15** Functional block diagram of the automatic titrator depicted in Fig. 13.14. (Courtesy of Ionics Inc.).

and about 8 gal of dilution water are used per month. The sample temperature must be between 1 and 40°C. Sample consumption is typically 10 cm³ per analysis, and suspended solids of up to 1/16-in diameter can be handled by the manufacturer's internal sampling system. The built-in self-diagnosing program is a major feature of this automatic titrator, which makes a precision metering device capable of making accurate and repeatable titrimetric determinations of the concentration of various acids and bases. It has been described as "a chemist in a box" but, according to its manufacturers, it is more than that as it is not subject to the human error or the judgement of the laboratory analyst.

Finally, it is worth emphasizing that titrators have progressed from the laboratory to process areas, where they are being applied to an increasing number of difficult problems involving on-line measurement and control. This trend is expected to continue as more and more laboratory instruments are being adapted for and applied to the chemical and petrochemical industries.

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# 14
## Automation in clinical chemistry

### 14.1 INTRODUCTION

Clinical analysis is probably the area best representing the evolution and gradual and systematic development of analytical automation.

In the past 30 years clinical laboratories have made increasing use of automated apparatus and accesories [1-3] (i.e. devices intended to replace or supplement human effort), chiefly as a result of the overload arising from the increasing attention paid to health, which in turn has resulted in an increasing number of analyses required for an increasingly larger number of patients. These needs have been met by an avalanche of commercial instruments automated to different extents and received with different attitudes by clinical workers depending on their skill, adaptation capabilities and usual needs (i.e *in vitro* or *in vivo*, intra- or extracellular analyses, etc.).

The reason why some clinical methods should be labelled automatic or not is not completely clear. The generic definition does not establish the extent to which human effort need be replaced or the method concerned be facilitated for the latter to deserve to be categorized as 'automatic'. A manual method is rarely considered automatic merely because it calls for a water-bath whose temperature is adjusted by means of a feed-back mechanism. On the other hand, self-contained continuous-flow instruments including the sampler, peristaltic pump, dialyser, spectrophotometer with flow-cell and recorder are common although, according to IUPAC's definition, improperly called 'automated'. In fact, few of the elements of such instruments, apart from the dialyser bath or the recorder servo, are regulated by a feed-back mechanism.

The methods typically used with high-throughput analysers involve processing biological fluid samples. Roughly 90% of all measurements are based on the molecular absorption photometric technique, while the rest are normally based on flame photometry and atomic absorption or, more recently, direct potentiometry with ion-selective electrodes. As a rule, these methods are not more automated than the manual counterparts that they replace and there is no strong argument for using a feed-back mechanism in the steps involved in the quantitative analysis.

The incorporation of computers into clinical chemistry has run in parallel with the automation and commercialization of complex analysers. The operation of multi-channel analysers is controlled by suitable software, as is the acquisition of analytical data. The earliest clinical analysers including computerized control of the analytical process were marketed in 1970. Virtually every analyser launched after 1980 is microprocessor-controlled.

The rapid growth of instruments with built-in microprocessors or computers has been the result of a number of reasons. First, the enormous growth of the number of clinical analyses required, which increased by 10-20% yearly between 1970 and 1980. Second, the need for better performance from analysers whose precision could not be improved or even preserved without computerization. Even the basic concept behind some analysers (e.g. centrifugal analysers) or enzymatic rate measurements with multi-detection are not feasible without  recourse to computerization.

Another pressing need fostering the incorporation of computers in clinical chemistry was the growing number, variety and complexity of STAT analyses, performed as fast as possible under conditions of serious risk to the patient's life. STAT assays cannot always be efficiently carried out manually. A special type of automatic analyser has been developed not to meet the need for a large number of analyses, but rather to ensure application of a variety of procedures with the minimum possible set-up.

It should be emphasized that microprocessors are relatively inexpensive accessories. All the above-mentioned reasons for the development of automation would not have been sufficient if the cost of the computerized control of the analytical process had not been so low and easy to repay.

It should also be noted that the most recent advances in automation, namely robotics, have also been incorporated into the clinical field, either by using conventional arms and adapting the remainder of the station (sofware, modules adapted to ELISA and microassays in general) as is the case with the system marketed by Hewlett-Packard, or by using robots specially designed for the clinical laboratory (e.g. the Biomek 1000 station manufactured by Beckman, whose arm is capable of performing up to eight simultaneous analyses by means of as many pipettes). The recent advent of these systems does not allow one to assess the advantages and disadvantages of their use in Clinical Chemistry. However, Craig has evaluated the advantages of automation over manual work in a clinical laboratory [4] and drawn the following conclusions:

(a) As far as quality control is concerned, automation can improve the quality of clinical tests because it is typically about four times more precise than manual procedures. With greater precision, automated procedures reduced quality control and the number of test repeated and run in duplicate

to a third of the former level at the six small hospitals where this was mea-
sured (Fig. 14.1).
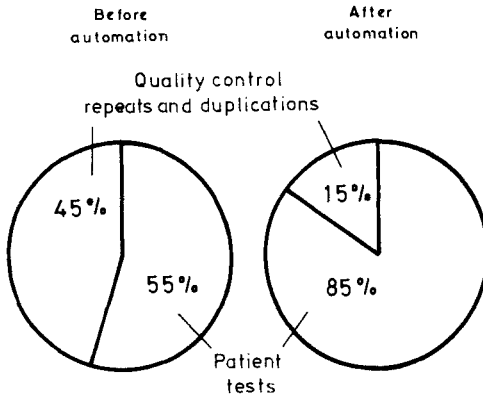


**Fig. 14.1** Reduction of quality control, repeats and duplications re-
sulting from automation. (Reproduced from [4] with permission of Fran-
cis & Taylor).

(b) Regarding the impact on labour productivity, automation can increase
it by 93% to as much as 472% according to the results obtained by Craig in his
study of selected hospital clinical laboratories [4]. By increasing the produc-
tivity of the existing staff, automation reduces the need to add personnel as
the laboratory's workload increases. It also improves the workload distribu-
tion and allows the laboratory to use less skilled people to process tests.
This frees skilled technologists for more demanding tasks. Space saving is
another parameter to consider when evaluating automation. By reducing bench
and chemical storage space, automation can reduce the size of the laboratory
or offset the need to expand it with an increasing workload. This results in
major costsavings as most laboratories operate on a cost per square foot
basis.

(c) As regards the financial impact on the hospital system, automation not
only increases laboratory efficiency, but also benefits the entire hospital
system. Thus, it accelerates laboratory turnround times, which can increase pa-
tient turnover, and it improves out-patient services by providing faster and
more timely diagnostic information. These benefits are most evident in small
hospitals, where it is easier to isolate the impact of automation on the hos-
pital system.

## 14.2 STAGES OF CLINICAL ANALYSIS

The analytical data required by a physician to make a diagnosis of a pa-
tients's state are the result of the various stages shown schematically in the
flow diagram in Fig. 14.2. After examining the patient, the physician requests
a series of analyses —either directly or via the computer, depending on the
type of hospital—, which leads to the subsequent stage, namely collection of
the patient's sample. This, after incubation and/or pretreatment, is placed on
the sampler via which it is introduced into the analyser which, after measure-
ment, processes data to deliver the corresponding results via a printer. A
built-in microprocessor usually controls the sampler functioning, acquires
data from the analyser and processes them for presentation via the printer.
The printed results can reach the physician in two ways: directly or through
the computer, depending on how the analyses were originally requested. The
data can be incorporated into a bank if there is one, and be used in the
future for statistical analysis of the evolution and overall state of differ-
ent events (e.g. frequency and evolution of a given disease, age, sex and
circumstances usually involved). Another contact between physician and patient
allows the latter to know his or her diagnosis.
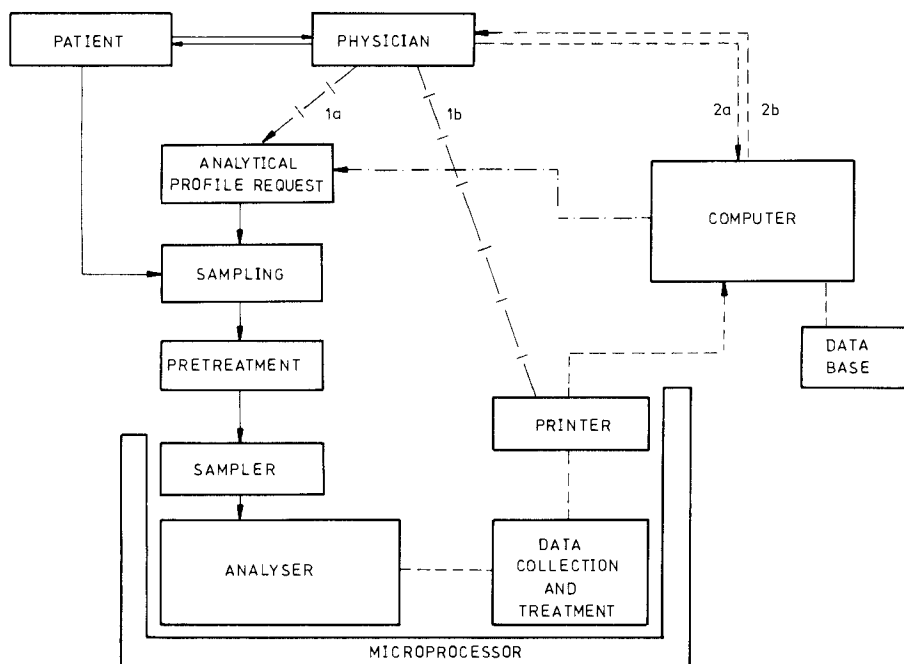


**Fig. 14.2** Scheme of the stages preceding a medical diagnosis (see
text).

The most distinctive features of each of these stages are commented on below.

### 14.2.1 Analysis request and sample collection

The instrument devoted to sample collection can be flexible for adaptation to the patient's needs —whether he or she is in bed or not— or be fixed in the laboratory. Manual sample collection obviously involves more personnel in this stage (the physician, the nurse or specialist effecting the collection, a technician, a clerk), which is remarkably simplified by the use of a computer. The work following collection should be perfectly organized in order to eliminate the risk of sample-patient mismatch, again minimized by the use of computers.

### 14.2.2 Sample storage, volume and pretreatment

Sample treatment should be considered part of the analysis program. Some instruments require blood samples that are to be aspirated by the sampling probe to be haemolysed, whereas others simply need centrifuged samples.

When the sample is to be stored for some time, the storage conditions should be appropriate to prevent any changes in the fluid composition. Unfortunately, these conditions are not clear-cut and this is an experimental area where industry can co-operate with the user to a great extent. The literature on this topic is frequently contradictory. Thus, serum and plasma from which red cells have been separated should be protected from light and evaporated; however, their refrigeration may pose more problems that it may solve as the precision of sampling is generally affected by temperature changes.

The sample volume should be small, although sufficient to perform all the assays required. The maximum amount of sample to be extracted from critical patients and children is 1 mL. This should be divided into parts sufficiently large to allow the different analyses required (e.g. glucose, $CO_2$) to be carried out. The chief problems resulting from the use of small sample volumes arise from the lack of homogenization when the sample must be reconstituted or thawed after lyophilization and freezing, respectively.

The instrument should only carry out the requested tests and use the sample volume required. Some simple instruments allow the implementation of analytical procedures requiring as little as 20 µL of sample and featuring excellent precision, reliability and throughput. Improvements in this respect have been aimed at developing automated instruments performing functions similar to those carried out manually. A reasonable goal in this sense is the possibility of carrying out twenty freely selected assays with 100 µL of blood sample, which results in a typical sample volume of 5 µL. Such a small volume

poses the added problem of adapting the instrument's chemistry and reading accordingly. Thus, the usual sample to final volume ratio in serum analysis is 1:8. This means that only 40 µL of the final mixture should be measured in the appropriate detector.

Carry-over arising from the contact between samples, species and reagents should be kept below detectable levels. Alternatively, a computer can be used to compensate for mutual contamination. A logical and dual solution to this problem involves minimizing contact between surfaces by using disposable pipettes, cuvettes, etc., and carefully washing and drying re-usable labware between samples.

### 14.2.3 Measurement

Some measurements such as enzyme activities must, by definition, be made under strictly preset conditions. Those methods involving dichromatic measurements, excessive dilution of the substrate, erratic control of temperature or undefined or non-standardized temperatures, or non-preset zero time  may yield results consistent with those provided by standard methods, but be considered inadequate because of the lack of suitable material for calibration. Instruments used to implement these methodologies not following the prescribed procedures may be fatal to the patient's treatment.

Stable calibration is a major attribute of automated instruments. The principle behind calibration is simple: the instrument must be calibrated with a suitable material prior to any new analysis and later, periodically, by using control materials to evaluate the precision of the assays. If the results obtained with the control material suggest a decrease in the reliability of the analyses, these should be stopped and the instrument be recalibrated —this is generally a slow, costly operation. Automated instruments are self-checking and self-adjusting. If they fail at all, their alarm systems stop their functioning, preserve valid results and delete the rest.

Starting new analyses only after the previous ones have been completed is the logical and analytically correct *modus operandi.* However, laboratory workers rarely follow this golden rule, so that their working schemes involve some overlap, particularly when batch analysers are involved as these allow for sampling, reagent dispensing, dilution and mixing while a series of samples previously introduced into the instrument are being measured with complete independence.

The ideal analyser should be flexible enough to allow the user to define the parameters governing data acquisition. The start of measurements, the average number of measurements per data point, the interval between measurements and their number should be definable by the user according to the method to be

Implemented and the characteristics of the instrument and detector employed. In addition, the ideal analyser should offer the user a variety of calibration alternatives. For linear methods, the techniques involved should include: (1) the use of theoretically derived factors for conversion of the series of measurements to concentration units; (2) calibrations based on one or two points and the use of a suitable blank; (3) multiple calibration by linear regression techniques. In order to obtain the best-fitting calibration graph, non-linear chemi-immunoassay methods may require calibrations based on multiple points and point-to-point, log-log, spline, high-order polynomial and potential-function regressions.

### 14.2.4 Data acquisition

This stage involves the occurrence of an appropriate interface converting the signal provided by the analyser to one readily usable by the microprocessor. The data from the detection system can be very simple (e.g. a single absorbance, potential or current intensity in equilibrium methods) or a series of data obtained from each sample during the evolution of the indicator reaction in kinetic methods. In either instance, the data are compared with those of the calibration graph stored by the microprocessor in order to obtain the analyte concentration in the unknown. These final data are sent to a printer for presentation.

### 14.2.5 Errors

Quality control in clinical laboratories has traditionally been based on a standard deviation limit of ±2%. This leads to errors and a high frequency of misrejections —the result may fall outside the limits for statistical reasons even in the absence of error— particularly with multi-channel analysers. Misrejections increase enormously with the number of analytical observations or channels when the above-mentioned limit is used for quality control. There are multi-rule programs for quality control that allow misrejections to be minimized while being sensitive to real errors. In addition, a multi-rule system indicates whether the 'out-of-range' condition is due to a systematic or random error. Other programs developed for this purpose detect a preselected level of error for a known probability of misrejections. The application of advanced quality control systems gives the user the confidence that the instrument is operating under medically acceptable limits of error.

### 14.3 CLASSIFICATION OF CLINICAL ANALYSERS

The large variety of clinical analysers currently available can be classified according to two basic criteria, namely (Table 14.1):

(a) The type of determination for which they are conceived. An instrument can be designed for the determination of one or several given species or, alternatively, be adaptable for different types of determinations. These last instruments, in turn, can be capable of determining one or several parameters (species) in a simultaneous (parallel) or sequential manner.

TABLE 14.1

Classification of clinical analysers

| According to the type of determination | Specific | Single-parameter | | |
|---|---|---|---|---|
| | | Multi-parameter | | |
| | Flexible | Single-parameter | | |
| | | Multi-parameter | Parallel | |
| | | | Sequential | |
| According to the type of technique | Continuous | SFA | | |
| | | UFA | FIA | |
| | | | CDFA | |
| | Discrete | With final transfer | Centrifugal | |
| | | | Others | |
| | | Without final transfer | Ordinary cuvettes | |
| | | | Prepared supports | Cuvettes with reagents |
| | | | | Dry reagents |
| | | | *In situ* | |

(b) The type of technique to be used (continuous or discrete). Among the continuous techniques implemented on clinical analysers are both the classical air-segmented [5,6] and the unsegmented types —the latter include flow-injection analysis (FIA) [7,8] and controlled-dispersion flow analysis (CDFA) [5]. Discrete techniques in turn can involve the transfer of the reaction mixture from the cup where it is originally held to the measuring cell. These techniques are typically employed by centrifugal analysers [10] and a large group of other analysers in which measurements are carried out in ordinary cuvettes, whether disposable or not, once the analytical reaction has been completed in another cell, vessel or tube. Techniques without final transfer include both analysers with ordinary cuvettes designed for the analysis of a large number

of samples, *in situ* analysers and those using reagents packed in columns or deposited on films —specially suited to the fast analysis of a few samples (STAT analysis).

Below are described some of the most representative clinical analysers according to the type of technique that they use, although due mention is made in each instance to the type of determination for which they are intended.

### 14.3.1 Continuous analysers

Continuous-flow analysers in their segmented version prevailed in the automation of clinical chemistry for over a decade, until they began to be displaced by batch analysers which, oddly enough, where introduced by the same manufacturers who launched and popularized SFA. This type of analyser was commented on in Chapter 5, which described in detail the Analyzers II, SMA 12/60 and SMAC, as well as a series of non-clinical applications grouped according to the type of detection system used.

The SMAC and SMAC II represent the highest degree of sophistication of SFA as they include a computer controlling the different functions, namely sample identification, sampling, pumping, detection and data acquisition, processing and delivery. Their incorporation in large hospital centres has resulted in more affordable workloads on account of the large number of samples that can be analysed for up to 20 parameters in a relatively short time.

Typical examples of the clinical applications of these instruments are the enzymatic and non-enzymatic determination of cholesterol in serum with the aid of the SMAC.

The non-enzymatic determination of cholesterol developed by Burchard [12] is based on its reaction with strong acids as described by Liebermann [11]. The method was formerly developed in its manual version by Huan *et al.* [13] and later automated by Levine *et al.* [14]. The reaction sequence taking place after the serum sample is mixed with sulphuric acid involves a colour change from red to violet (bis-cholestadienylmonosulphonic acid, BCDMSA) and then to green (bis-cholestadienyldisulphonic acid, BCDDSA), which is monitored at 630 nm:

Cholesterol + $H_2SO_4 \rightarrow$ BCDMSA
BCDMSA + $H_2SO_4 \rightarrow$ BCDDSA
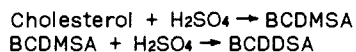
Figure 14.3 depicts the manifold used by the SMAC. The reagent, sample and some air are aspirated by a peristaltic pump into a single channel. The above-mentioned reaction takes place at reactor $R'_1$. The heat released in this exothermic reaction is absorbed by a heat exchanger located prior to the flow-cell. Finally, the reaction product is sent to waste by aspiration. The chief

interferences with the method are posed by amphetenzine B, bilirubin, haemoly-
sis, lipaemia, lipochrome and tryptophan.
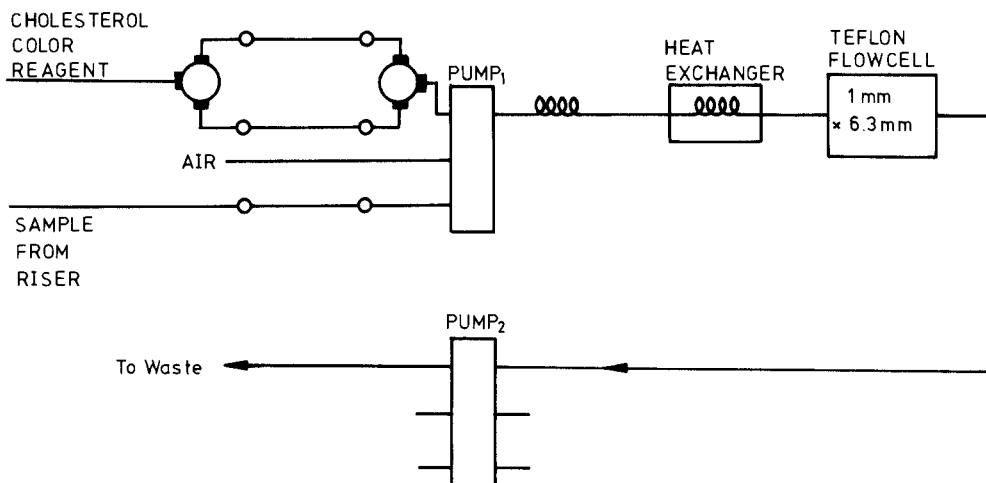


**Fig. 14.3** Manifold for the non-enzymatic determination of cholester-
ol. (Courtesy of Technicon).

The enzymatic method for determination of cholesterol is based on that of
Klose *et al.* [15], as modified by Leon and Stasiw [16]. It involves the use of
cholesterol stearase to hydrolyse the cholesterol esters in serum to free chol-
esterol, which is oxidized to $H_2O_2$ that in turn forms a quinoneimine dye. The
reaction is quantitative, so the concentration of the dye formed is directly
proportional to that of cholesterol in the sample. Figure 14.4 illustrates the
function of the SMAC channel used for the determination, in which the reagent
stream (cholesterol oxidase, cholesterol stearase, peroxidase, phenol and 4-
aminophenazone), the sample and some air are aspirated by the pump and, after
mixing in reactor $R_1$, are incubated for 4 min in a bath at 37°C, after which
the dye is extracted into alcohol and sent to the detector, where its absor-
bance is measured at 525 nm. The aqueous phase from the extraction and the
cell waste are aspirated by pump $P_2$. The method has fewer and less serious
interferences than its non-enzymatic counterpart.

Radioimmunoassays (RIA)[1] can be implemented on some commercially available

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[1] In RIA, the analyte is the antigen in the antigen–antibody reaction and
there is competition between a fixed concentration of reactive labelled an-
tigen and non-labelled antigen in the sample. The measured (counted) species
can be the antibody (and the bound antigen, both radio-labelled and non-la-
belled) after separation of the non-bound antigen or, alternatively, the
non-bound radio-labelled antigen. The results are obtained from the cali-
bration graph of antigen concentration *vs.* bound (or free) radioactivity.

automated segmented-flow analysers such as the Technicon STAR, whose hydraul-
ic system is controlled by a microprocessor as in the SMAC. The method imple-
mented in the STAR is based on the automatic separation of the free and bound
antigen through the covalent binding of the antibody molecules to an organic
polymer containing ferric oxide. A computer-controlled magnetic field entraps
the solid-phase antibody, while the free antigen is washed to waste. As the
magnetic field is turned off, the solid-phase antibody particles wash through
a flow gamma counter, where the antibody-bound radio-labelled antigen is
counted.
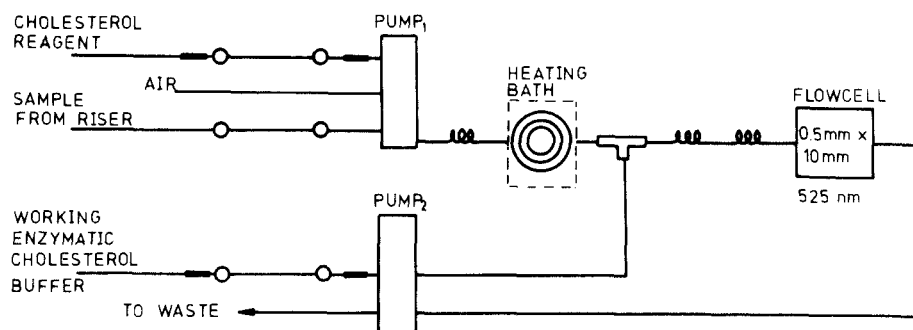


**Fig. 14.4** Simplified scheme of the air-segmented manifold used for
the enzymatic determination of cholesterol. (Courtesy of Technicon).

The method requires the addition of labelled antibody and antigen to the
stream containing the sample alone and the pumping and recycling of the buffer
solution in the absence of the sample, which is aspirated in 100-$\mu$L volumes
under the computer's pneumatic control at a rate of 60 samples/h. The radio-
labelled antigen and the solid-phase antibody are linked to the analytical
stream by two hydraulic circuits provided with pinch valves that return the
reagents to their containers when not needed. Error detector assemblies for
the sample and reagents allow convenient checking. After mixing, the sample,
radio-labelled antigen and solid-phase antibody are circulated through a coil
at 37°C for 10 min.

Completion of the incubation time is detected by the LAS error-detector as-
sembly, which monitors the analytical stream at the outlet of the incubator.
The microprocessor activates the magnets and, as stated above, the solid-phase
antibody is trapped while the free antigen washes through. The pinch valve is

then activated, directing the free antigen through a resistant coil to waste. A buffer solution flows into the analytical stream from above the first magnet and washes the bound antigen. Then, the first magnet is switched off. The antibody then flows to the second magnet, where it is trapped and washed again. When the second magnet is turned off, a vibrator is activated to dislodge the antibody particles. The pinch valve is activated to direct the analytical stream through the coil in the scintillation detector. The computer accumulates counts for 45 s.

The calibration graph is fitted by a log–log linear regression. The calculated concentration for each standard is compared with its assigned value, the graph being rejected if there is excessive divergence.

Flow-injection analysis, whose foundation and applications are described in Chapter 6, is of great relevance to clinical chemistry —both to classical continuous and batch methodologies. Its potential in the determination of one [8] or two [17] components at a remarkably high rate with low consumption of sample and reagents has been demonstrated ever since the beginning of this revolutionary methodology. The variety of problems solved by FIA have been described in various reviews on the FIA-clinical chemistry association [18–20]. Current FIA trends in this field point to multi-analysis, still in a developing stage but with such potential that it might turn FIA into the most suitable alternative to existing methodologies on account of its inherent advantages commented on in preceding chapters. The methods —both enzymatic and non-enzymatic— described below illustrate its simplicity and versatility.

The non-enzymatic determination of creatinine in urine and serum developed by van Staden [21] is based on Jaffé's reaction [22,23], which involves the formation of a red-orange compound on interaction of the analyte with picric acid in an alkaline medium. The manifold used is shown in Fig. 14.5. The sample —deproteinized serum to avoid the interference from proteins or urine diluted to 1:100— is placed on a sampler from which it is injected into the carrier and merged with a basic stream of picric acid. The indicator reaction takes place along a reactor submerged in a bath thermostated at 35°C, after which it reaches the detector flow-cell, where the absorbance is monitored at 520 nm. The results obtained by this method are consistent with those found by the standard kinetic method [24,25]. The sampling frequency is 120 h$^{-1}$.

Urea can be determined enzymatically by two FIA methods with potentiometric detection and different concepts. The first of such methods was devised by Ruzicka *et al.* [26] and is based on the oxidation of the analyte with urease, which involves the exchange of protons with the medium. Provided that the carrier is continuously buffered, the pH measured and the urea content in the serum sample are linearly related. The straightforward manifold used is de-

picted in Fig. 14.6. A buffer solution containing the enzyme is propelled con-
tinuously through the system by a peristaltic pump and receives the injected
serum sample. The reaction, taking place in a reactor thermostated at 40°C,
results in a pH change in the sample zone which is detected upon passage
through the glass ring electrode located in series with the reactor. The calo-
mel electrode acting as a reference is located in the waste cup, whose liquid
level is maintained by aspiration. The sampling rate is 60 samples/h and the
enzyme consumption, normally 25 units per analysis, is substantially decreased
by using the merging zones mode.
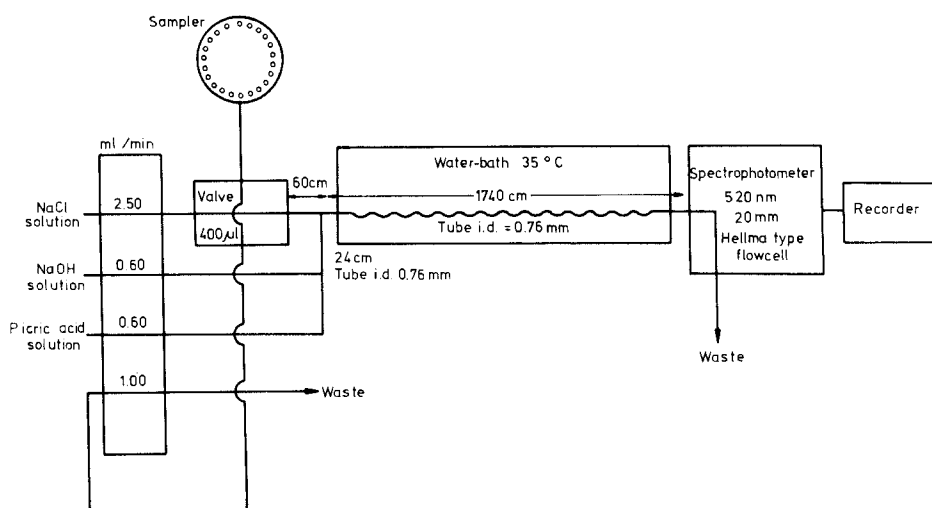


**Fig. 14.5** Flow-injection configuration for the non-enzymatic deter-
mination of creatinine in biological fluids. (Reproduced from [21]
with permission of Springer-Verlag).

The method developed by Gorton and Ogren [27] is a representative example
of the use of an on-line separation technique (dialysis) and an immobilized en-
zyme reactor (Fig. 14.7). The serum or urine sample injected into the donor
stream is transferred to the acceptor stream on passing through the dialyser.
The reaction takes place in the enzymatic reactor, and the ammonia formed is
sensed by a selective electrode. The lack of interferents, its wide determina-
tion range, high sampling frequency (80 h$^{-1}$), simplicity and low cost per ana-
lysis make this an interesting method.
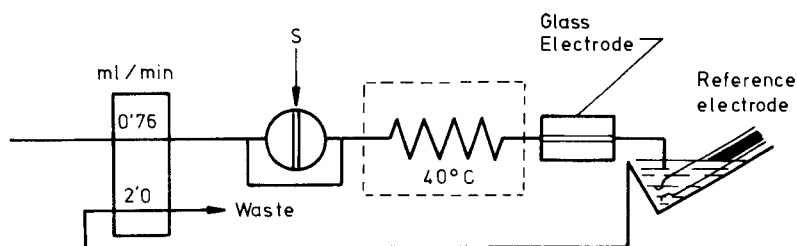
**Fig. 14.6** Single-channel FIA system for enzymatic determination of urea based on potentiometric (pH) measurements. (Reproduced from [26] with permission of the American Chemical Society).

The 'controlled-dispersion flow analysis' mode proposed by Riley *et al.* [9], the foundation of which was commented on in Chapter 7, has been applied in various methods used in the clinical field. The manifolds shown in Fig. 14.8 were used in developing determinative methods for triglycerides and theophylline (Fig. 14.8a and b, respectively) [28]. The determination of triglycerides is based on their hydrolysis to glycerol and fatty acids by lipase. The glycerol rleased is subjected to a reaction sequence yielding a coloured compound ($\lambda_{max}$=503 nm) as a result of the oxidation of p–iodonitrotetrazolium violet (INT) by the reduced form of $NAD^+$ in the presence of the enzyme diaphorase:

$$\text{Triglycerides} \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acids} \tag{1}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{kinase}]{\text{glycerol}} \text{glycerol-1-phosphate} + \text{ADP} \atop \text{(G1P)} \tag{2}$$

$$\text{G1P} + NAD^+ \xrightarrow[\text{dehydrogenase}]{\text{G1P}} \text{dihydroxyacetone-phosphate} + \text{NADH} \tag{3}$$

$$\text{NADH} + \text{INT} \xrightarrow{\text{diaphorase}} NAD^+ + \text{INTH} \atop \text{colourless} \qquad\qquad \text{red}$$
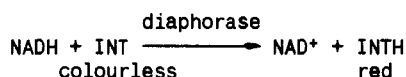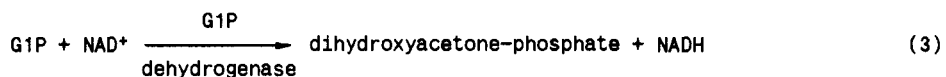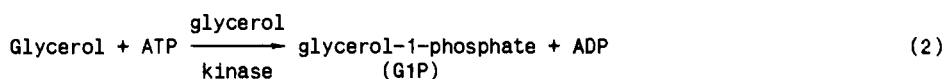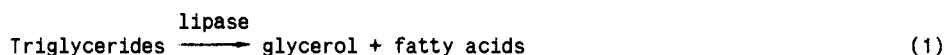
The sample and reagent probes are transferred from their respective ves-

sels to those of the carrier. The identical length of the sample and reagent
tubes facilitates their simultaneous arrival at a T-junction. When the react-
ing plug reaches the detector, the pump is stopped to measure the reaction
rate and then re-started at a high rate to flush the system with de-ionized
water. The accurate control of the stop and go of the peristaltic pump makes
this technique suitable for the stopped-flow mode.
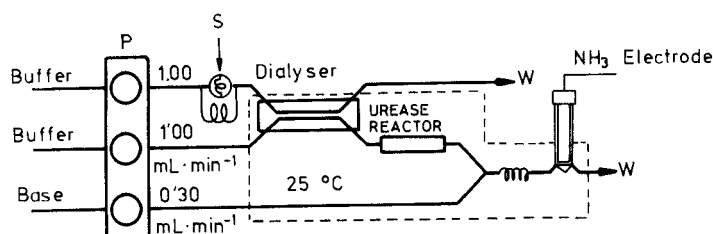


**Fig. 14.7** System for the enzymatic potentiometric determination of
urea including a dialyser and an immobilized-enzyme reactor. (Repro-
duced from [27] with permission of Elsevier).

The manifold used for the determination of theophylline is similar, but
includes another reagent channel. The immunoassay involves the presence of the
coenzyme $NAD^+$, the reduced form of which is used to monitor the reaction
($\lambda_{max}$=340 nm). As the antibody and bound theophylline cannot be held in the
same reservoir, both are mixed *in situ* by means of a merging zones system.
Figure 14.8b shows the sampling position; the probes are kept in the carrier
solution during the rest of the cycle. The stopped-flow mode is also applied
in this case. As in the previous determination, 10 µL of sample and 120 µL of
reagent are sufficient for each assay.

The instruments with which continuous techniques of clinical analysis are
carried out belong to the group of flexible analysers. Segmented-flow instru-
ments used in this field are multi-parameter and allow the simultaneous deter-
mination of the different species by means of systems splitting the aspirated
sample into as many lines as parameters are to be determined (see Chapter 5).
The adaptation for analysis of a new parameter is readily accomplished by
simply changing the corresponding analytical cartridge. Thanks to its versatil-
ity, the FIA technique is adaptable to any type of analysis, whether for one

or several species, in a simultaneous or sequential manner. Adaptations are usually simple and only require replacement of the reactor for another of the appropriate length and increasing or decreasing the number of merging points, depending on the chemical systems involved —replacing and replaced. Finally, CCDF is adaptable to any type of analysis, although it has only been applied to fairly simple determinations so far.
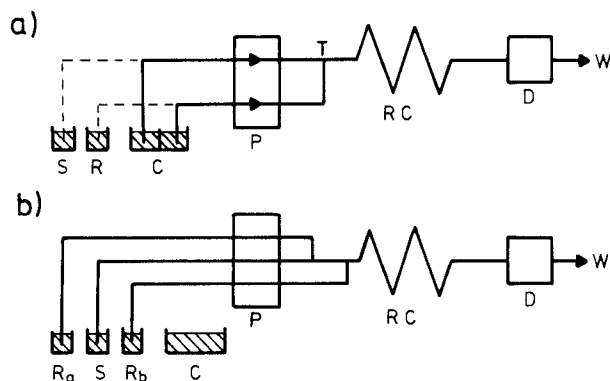


**Fig. 14.8** Controlled-dispersion flow analysis manifolds for determination of (a) triglycerides and (b) theophylline. (s: sample; R: reagent; c: carrier; P: pump; T: connector; RC: reaction coil; D: detector; w: waste). (Reproduced from [28] with permission of the Royal Society of Chemistry).

Continuous-flow techniques offer different advantages on application to clinical chemistry. The differences between one another are most evident when they are applied to the determination of the same analyte. Thus, let us consider the determination of albumin by all three techniques. The Bromocresol Green (BCG) method proposed by Rodkey [29] is specific for this substance. At pH 4.5, BCG binds to albumin to form a stable complex which is monitored at 630 nm. The corresponding SFA method is fairly fast (150 samples/h) and precise, and has few interferences —chiefly from ferrin and some proteins at high concentrations. The problem of the sample turbidity or the presence of lipaemic substances is minimized by increasing the sample dilution to 236:1. The manifold used for this determination is shown in Fig. 14.9a.

The FIA method proposed by Mindegaard [30] for this analyte uses the merging zone mode (Fig. 14.9b), so that the reagent plug completely merges with that of sample if this is used in diluted form or only with its trailing portion if it is used in concentrated form. Hence, the same extent of dilution

can be used for all samples without the risk of any falling outside the absor-
bance range yielding the minimum error. The sampling rate is 300 samples/h and
the reagent consumption is 15 µL per assay (i.e. barely 0.4% of the amount
needed in the standard manual method). Because of its kinetic nature, the
method has fewer and less serious interferences than its equilibrium
counterpart.

The CCDFA method for albumin involves the use of the manifold depicted in
Fig. 4.9c. The reagent probe is kept continuously in the reagent reservoir
(R). At the start of the cycle, the sample probe is separated from the carrier
solution (de-ionized water), C, and taken to the sample cup, S, by a turn of
its arm, thereby flushing the sample. The pump is then stopped and the probe
returned to the carrier solution. After re-starting the pump, the sample plug
is transported to the reaction coil, where it meets the reagent. The reaction
mixture subsequently reaches the photometric detector, where it is monitored,
and is sent to waste. The method uses 240 nL of sample and 360 µL of reagent.
It features a sampling frequency of 120 $h^{-1}$ and, like the FIA method de-
scribed above, has few interferences because of its kinetic nature.
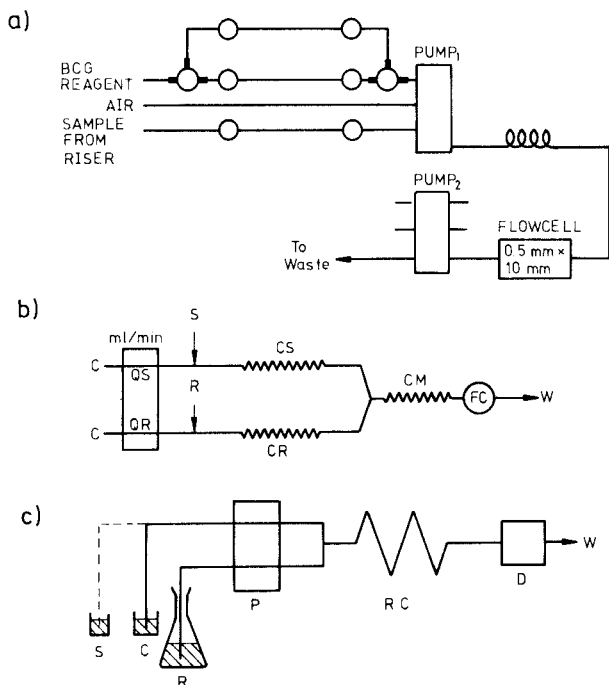


**Fig. 14.9** Manifolds for the determination of albumin by the Bromo-
cresol Green method, carried out by three different continuous-flow
techniques: (a) SFA; (b) FIA and (c) CDFA. (Courtesy of Technicon and
reproduced from [30] and [28] with permission of Elsevier and the
Royal Society of Chemistry, respectively).

In short, FIA features the highest sampling rate and involves the least sample manipulation as no successive dilutions are required. On the other hand, CDFA uses the least amount of sample and reagents and SFA allows the simultaneous analysis for another 19 parameters —FIA affords only 2-3 parameters and CDFD only one.

### 14.3.2 Batch analysers

The essential feature distinguishing these analysers from their continuous counterparts is the fact that the sample preserves its integrity during the process as a result of being held in the reaction cup (also the measurement vessel in analysers without final transfer), isolated from the rest of the system. To this group belong a variety of instruments, from the multi-parameter analysers conceived for the analysis of a large number of samples and parameters in a short time (e.g. Centrifichem, RA-1000) to those affording the determination of a single species in each sample (ICA 113).

These analysers are less prone to carry-over than continuous-flow analysers. In addition, the allow the use of strong acids and/or organic solvents. However, they do not permit the incorporation of continuous separation techniques. The existence or absence of transfer from the reagent addition vessel to the measuring cell allows them to be classified into two large groups: with and without final transfer.

#### *14.3.2.1 Batch analysers with final transfer*

These, in turn, can be classified into 'centrifugal' and 'non-centrifugal' analysers.

*Centrifugal analysers* comprise the so-called 'third generation' of automatic analysers. The chief components of a centrifugal analyser, namely the sample tray and the transfer disc, are depicted in Figs 8.6 and 8.7. The capacity of the sample tray  ranges between 15 (Rotochem II) and 36 cups (Rotochem IIa and Rotochem CFA 2000). The tray can be re-usable (e.g. the three above-mentioned analysers plus the Centrichem 600 and the Gemsaec) or disposable (Gemin, Flexigem and Multistat). The principal quality of centrifugal analysers is the simultaneous sensing of blanks, standards and samples by means of a single detection point. The detection techniques most frequently used by these analysers are photometry and turbidimetry —some, such as the Multistat III FS/LS, also afford nephelometric and fluorimetric sensing. The use of centrifugal analysers has grown enormously over the last decade. They are usually applied to kinetic measurements, which are handled by a variety of software, from programs including two-time measurements to multi-measurements with preselectable measurement times and intervals. According to the classification established in Table 14.1, these are flexible, single-parameter instruments.

A representative example of a flexible, single-parameter analyser with final transfer is the Vitatron Akes, depicted in Fig. 8.5. In the aspiration position, the sample meets the reagent or diluent stream and the reaction mixture is subsequently transferred to the measuring cuvette, from which it is flushed by the aspiration system after detection, the cuvette being suitably washed. The instrument includes a linear sample train, sample turntable, sampling head, dilutor, reagent dispenser, data-input keyboard, photometric detection system, computer, printer, evacuation pump and wash solution doser. It is prepared for kinetic measurements.

Some of the commercial instruments available for enzyme-linked immunospecific assays (ELISA)[2] fall into this group of non-centrifugal analysers with final transfer, while others belong to the category of analysers without final transfer. Among the former are the Behring ELISA, a photometer marketed by Behringwerke, and the Allergo-zym Atom-336 made by Biotron. Both require the prior incubation of the microtitration plates until the formation of the monitored coloured product, the liquid on the plates then being transferred to the
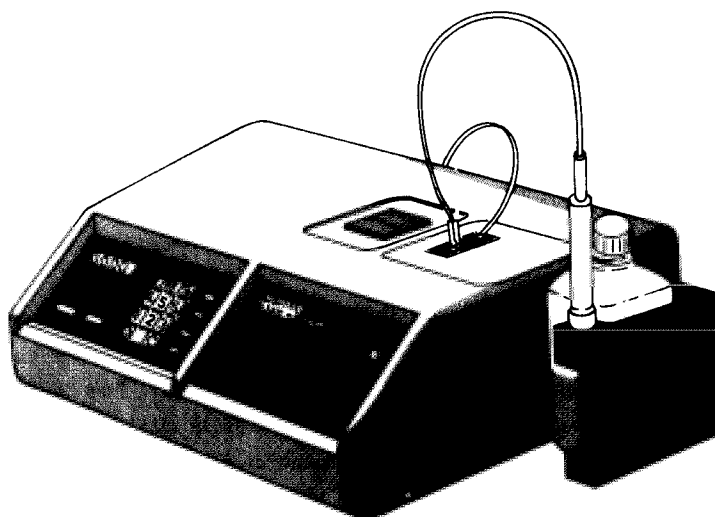


**Fig. 14.10**    Behring ELISA photometer. (1)  Lamp protective cover; (2) flow-cell; (3) manual aspirator; (4) waste bottle; (5) cuvette holder; (6) display;  (7) keyboard; (0) filter  compartment. (Courtesy of Behringwerke).

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[2] In  this technique, the detection of the  antigen (analyte)-antibody complex is  carried out through enzymes bound to one or the other component. The enzyme is  sensed through  the changes  observed in  the substrate  added. The enzymes commonly chosen induce colour changes in the substrate.

photometer cell for measurement of the absorbance. The measured absorbance is used to calculate the concentration of the unknown protein, which is displayed on a screen.

Figure 14.10 shows the appearance of the Behring ELISA photometer. It is a single-beam instrument with a halogen quartz lamp, interchangeable interference filters, keyboard and screen, which uses flow-cells of 1 cm light path. The assembly is controlled by a microprocessor and can work with or without a printer and be optionally linked to a computer or a laboratory management system. The sample, after incubation on a plate, is aspirated manually to the flow-cell, where it is measured and then sent to waste, the cell being washed before receiving a fresh sample.

The sequence of events preceding the measurement is as follows: in the sandwich assay, the sample is placed on the plate and the free antigen contained in it reacts with the excess of antibody present on the tube walls. The non-specific components of the sample and the potential interferences are eliminated by washing, after which the peroxidase-conjugate antibody binds to the free antigen determiners in a second reaction. The excess of conjugate antibody is then removed by washing and the activity of the bound enzyme is determined. The enzymatic conversion of hydrogen peroxide is halted by adding dilute sulphuric acid, after which the colour intensity, proportional to the antigen concentration in the sample, is determined.

In the assay, the antigen and enzyme-conjugate antigen compete for the antibodies fixed to the tube walls. The free components are removed by washing. Then the enzymatic activity of the bound enzyme is determined. The antigen concentration in the sample is inversely proportional to the colour intensity. The concentration of the analyte in question is calculated on a polygonal calibration graph or by calibration with two points. The instrument affords kinetic measurements.

### 14.3.2.2 *Batch analysers without final transfer*

There are a variety of instruments involving the transfer of the sample to the measuring cell prior to measurement. Such instruments are used to implement a variety of techniques, some of which are applied *in situ*, while others involve the use of ordinary cuvettes —either disposable or not— or prepared supports such as reagents packed or forming dry films.

In the so-called *in situ* techniques, the sample, alone or mixed with the reagent, is placed in the measuring cell by direct aspiration or addition. The overall analysis time is the same as that of measurement insofar as the preliminary separation, incubation and transfer stages are all eliminated. The instruments used to apply this type of technique are particularly useful for

emergencies. Their chief limitation is their inapplicability to kinetic meth-
ods —especially the slower ones. Below are described some of the instruments
belonging to this group.
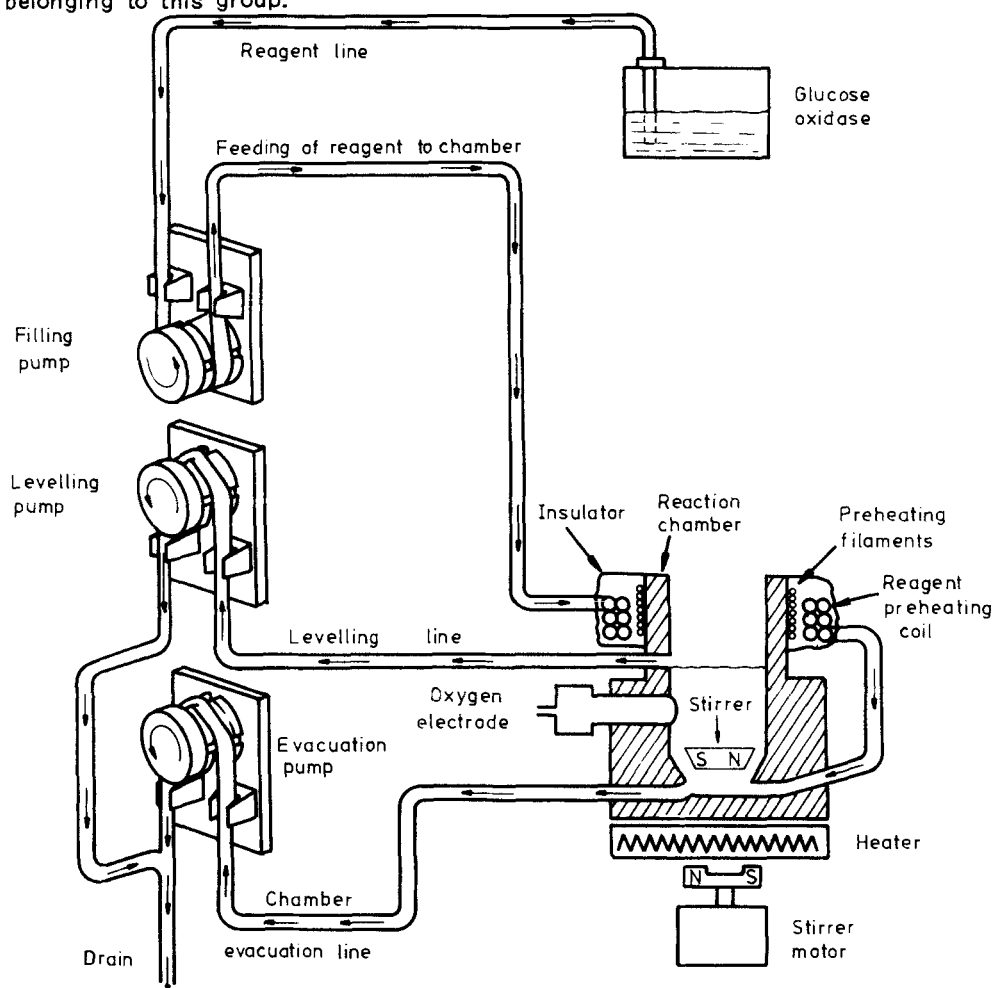


**Fig. 14.11** Manifold of the ASTRA 8 single-parameter module. (Courtesy
of Beckman Instruments).

Typical representatives of *in situ* operating instruments without final
transfer are those of the ASTRA (Automated STAT/Routine Analyzer) series (Mod-
els 4 and 8), marketed by Beckman and featuring four or eight measuring cuv-
ettes. They allow programming of a sequence of up to 80 samples with their cor-
responding identification number and assay required at a rate of 70–85
samples/h and with a consumption of 8–50 µL of sample for the different as-
says. They use both photometric and potentiometric detectors. Routine analyses

can be halted at any time to perform emergency assays. Figures 14.11 and 14.12 show a single-parameter (glucose) and a two-parameter (Na and K) module, respectively. The former requires the use of three single-line peristaltic pumps. The one at the top of functions to transport the reagent, after heating, to the reaction chamber; the other two level and evacuate the measuring chamber, where the sensing system (an oxygen electrode) is located. The determination of Na or K requires the presence in the measuring cell of both a buffer and a conditioner and hence a new pump to propel the reagents to the cell, at the inlet of which is the reference electrode, while the indicator electrodes face each other at both sides of the cell. The levelling and evacuation lines coincide with those used in the determination of glucose. In both in-
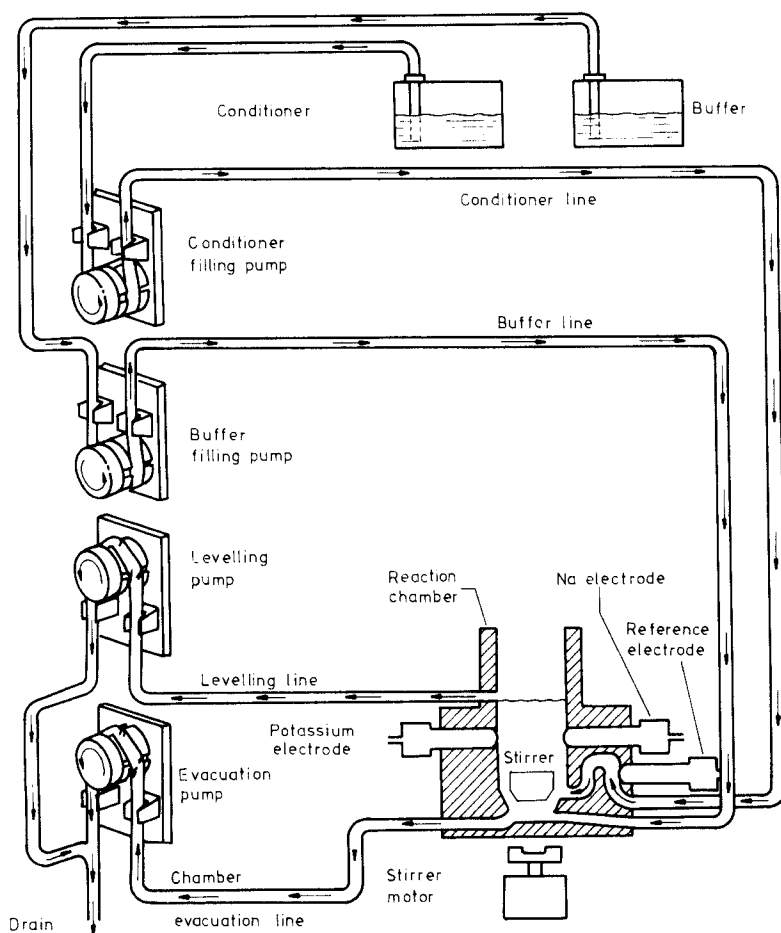


**Fig. 14.12** Manifold of the ASTRA 8 two-parameter (Na/K) module. (Courtesy of Beckman Instruments).

stances, sample and reagents are mixed and homogenized by magnetic stirring. The sample is aspirated from the sampler into the measuring cell by means of a probe. The pumps used do not require perfectly reproducible functioning insofar as the reagent volume placed in the measuring cell is fixed by the levelling line; neither this nor the evacuation line requires accurate flow-rates.

Blood gas analysers also belong to this category of *in situ* instruments and allow measurement of 2–4 different parameters in a blood sample by means of electrodes and cells connected serially with the line where the sample (typically 65–120 μL) is aspirated or injected. The presence of a valve in the line connecting the electrodes and cuvettes facilitates the calibration with gases for $pCO_2$ and $pO_2$ electrodes and with buffers for pH electrodes. The microprocessors built into these analysers can calculate the concentration of gases in blood allowing for *in vitro* temperature changes —yet, the accuracy of these adjustments is arguable on account of the lack of data on reference intervals or standard values of gas parameters in blood at temperatures other than 37°C or thereabouts. This type of instrument is represented by the IL System 1303 from Instrumentation Laboratory and the Models ABL 300, ABL 3, ABL 30 and ABL 4 (the last includes a potassium electrode for valinomycin), marketed by Radiometer.

The Radiometer ICA 113 is intended for the determination of ionic calcium. It is a specific, single-parameter batch analyser without final transfer operating *in situ*. It consists of a reservoir and aspiration, equilibration and measuring system, together with a washing and wasting system avoiding carry-over between samples (serum or plasma). It has several sample trays, so that one can be in the measuring stage while others are prepared for analysis. Each sample is identified on a print-out by the number of the tray where it is held and the number it occupies in the tray. The aspiration system consists of a pick-up arm with a nozzle taking accurately measured portions of sample. The first few portions taken are used to flush the system. The equilibration unit bubbles 5.7% $CO_2$ through water; the gas diffuses to the sample through the thin walls of a silicone tube. In this way, the $pCO_2$ of the sample is adjusted to approximately 40 mmHg, so that the resulting pH is about 7.4. Both this unit and the measuring cell are thermostated at 37°C. The working electrode has a heterogeneous PVC membrane and is protected from protein contamination by a readily replaceable Cellophane membrane. The instrument also features a reservoir, an aspiration probe and digital displays giving both the analyte concentration and the sample pH, a critical factor insofar as it influences the ionic calcium concentration. The relationship between both parameters is a key factor as normal ionic calcium values may be accompanied by anomalous pHs, or vice versa, and result in erroneous diagnoses if not controlled simultaneously.

Unlike anlysers using *in situ* techniques, those employing ordinary cuvettes usually have more than two cells, either disposable or not, in which a given parameter can be measured simultaneously or sequentially in several samples. These are general multi-parameter instruments adaptable to particular needs by introducing small changes, so that they are perfectly suitable for small laboratories requiring automatic analyses but unable to afford AutoAnalyzers.

A typical example of batch analyser without final transfer using ordinary cuvettes is the Technicon Model RA-1000. As can be seen from Fig. 8.10, it consists of a central unit with 100 disposable cuvettes receiving samples and reagents from two additional trays. A computer controls all the instrument functions, acquires data and presents the final results once processed. This analyser is suitable for both kinetic and end-point measurements and can perform emergency analyses without significantly altering its functioning.

Another instrument in this group is the DACOS [31-33], equipped with eight movable spectrophotometric detectors located in the centre of a carousel holding the measuring cuvettes, which is spun one turn every 6 s (Fig. 14.13). There is a tungsten lamp in the centre of the carousel serving as the light source of each optical channel, also comprising an interference filter and a detector. The design allows for rapid measurements and increases the flexibility of the analyser for adaptation to a variety of analytical procedures. Blank absorbances can be obtained before adding the reagents, and a second reagent can be added 2 s after the first. The instrument is suitable both for rapid end-point measurements and for slow kinetic methods requiring up to 10 min to monitor the absorbance change. The possibility of making measurements at up to eight different wavelengths allows side-reactions to be monitored, several analytes to be determined in the same sample and blank measurements to be made. In addition to the central carousel, the instrument has a sample and a reagent tray, in addition to a washing station, all of which are spun as programmed via the minicomputer used to dispense the required amounts of sample, reagent or wash solution into the appropriate cuvette by means of a probe. Once the reagent has been added, the probe vibrates in the reaction mixture to ensure efficient mixing. After measurement, the cuvette is drained and washed before the following analysis is started. Only those measurements required for the type of analysis selected by the user are stored by the computer, which consists of two modules: the control unit and the processor. The former includes the sampling, reagent delivery, incubation and measuring modules, and the latter is made up by the minicomputer, electronic circuitry and a printer. The sample cups are located on a carousel around that of the reaction cuvettes (32 cells in a system thermostated by means of a water

bath). The carousel takes 3.75 or 5.625 s for measurement of each sample, so that it turns around in 2 or 3 min as programmed. Sampling and the first reagent delivery are carried out by aspiration with a single probe that places both ingredients in the measuring cell. Addition of a second reagent requires an auxiliary probe programmed according to the method used to perform the insertion in the first turn of the carousel or at a later stage. Detection is performed by bichromatic photometry, which improves the reliability of measurements thanks to the blank self-measurement. End-point and reaction-rate measurements require a minimum of two or three turns of the carousel, respectively. Calibration can be performed with up to six different materials by linear regression for linear methods using multi-point calibration. Other mathematical routines are stored in the computer's memory for non-linear immunochemical methods or absorbance conversion factors in enzyme activity units for enzymatic methods.
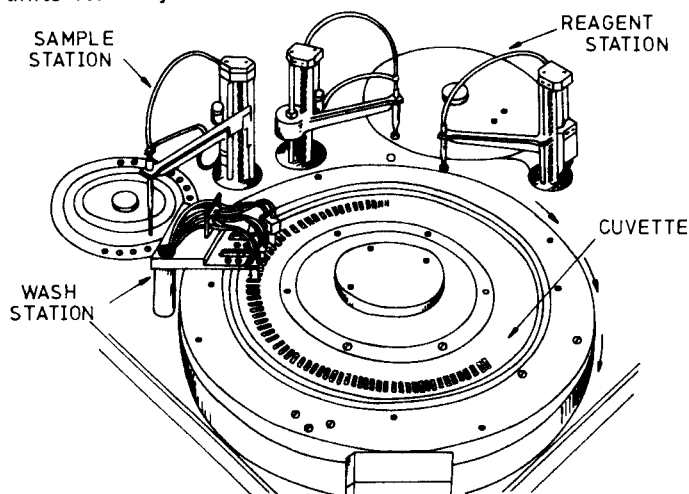


**Fig. 14.13** Scheme of the DACOS analyser. The rotary light source is located under the central cover. Around it spin at 0.6 rps eight interference filters for measurements at eight different wavelengths. (Courtesy of Coulter Electronics).

There are batch analysers without final transfer suitable for immunoassays, such as the ELISA Processor II·and the Behring Analyser Nephelometer, both manufactured by Behringwerke.

The ELISA Processor II is an instrument for automatic washing, reagent dispensing, quantitative photometric determination and result analysis in enzyme immunassays with microtitration plates. As can be seen from Fig. 14.14, it consists of a dispensing unit, reagent station, photometer, washing and dosing station, transport system, keyboard, screen and printer.
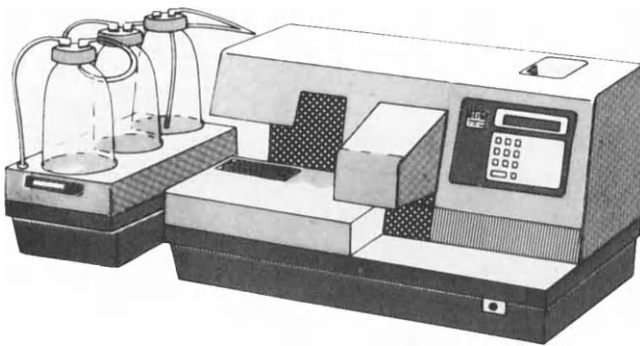
**Fig. 14.14** Front view of the ELISA Processor II. (1) Dispensing unit; (2) reagent station; (3) photometer; (4) washing and dosing unit; (5) transport frame; (6) screen; (7) keyboard; (8) printer; (9) on; (10) off. (Courtesy of Behringwerke).

The dispensing unit functions to deliver and drain wash solution and ensures the vacuum needed (approximately 0.6 bar) for aspiration.

The reagent station holds all the flasks required for the Enzygnost (R) test, in addition to a further reagent for specific assays. It can hold up to six reagent flasks, of which at least two are for the conjugate an one is for distilled water.

The transport system receives the microtitration plate (twelve rows of eight small cups each) and drives it linearly under the washing and dosing unit, which consists of two combs of sixteenth teeth each (two rows of eight teeth) simultaneously filling and emptying the cups in a row, and a moving reagent dispensing tip consisting of a single-line system provided with a 2.5 mL syringe connected to the different reagent flasks through a step valve.

The photometer effects bicromatic measurements by the appropriate combination of two of the five filters available. The radiation from the light source (a halogen lamp with a reflector) is parallelized almost completely and passed through the filter wheel, which splits it into eight beams by means of an eight-arm conductor. These beams traverse simultaneously the eight cups of one row in the plate from bottom to top. Eight photodiodes located over the cups measure the transmitted light.

The units controlling the two functional assemblies (washing and dosing on the one hand and detection on the other) are separated from each other and

linked via an internal interface. The reagent lack detector and the sensor acknowledging the units of a divisible plate work in an analogue fashion, as do the microtitration plate and the filter wheel. All the analogue signals, in addition to that generated by the photometric detector, are converted into digital signals of proportional frequency. The result of the logarithmic treatment of the data is delivered through a printer, as are occasional warnings (e.g. out-of-range measurements), malfunctions and errors.

The operational sequence of the instrument is as follows: during the first incubation, the antibodies (or antigens) present in the sample bind to the antigens (or antibodies) fixed to the cups of the microtitration plate. The enzyme conjugate is added after removal of unbound reactants by washing. After a second incubation the conjugate is eliminated and the substrate is dosed. The enzyme activity results in the appearance of a coloration, the development of which is halted by a braking solution after the substrate incubation has finished, after which the plate is taken to a position close to that for measurement by the photometer and the cover is replaced. In this position is measured a blank, from which the specific channel data are computed. Then the twelve rows of the plate are positioned to be measured and the eight  cups of each row are measured simultaneously. A first 'measurement in the dark' performed by blocking the light beam allows the influence of stray light and the temperature on the instrument electronics to be determined.
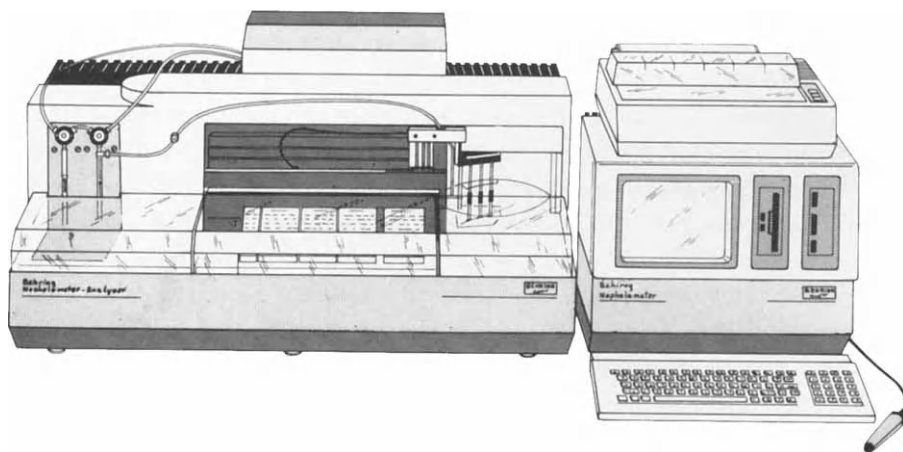


**Fig. 14.15** Front view of the Behring Nephelometer Analyzer (sample analyser and computer terminal monitor). (Courtesy of Behringwerke).

After placing the specific interference filters required in the beam trajectory, measurements are taken to calculate the corresponding molar absorptivities, which are proportional to the transmitted light intensities and to the stored reference values. While the plate is still being measured, the results from the already measured cups are computed from the three measurements performed per cup and the specific correction factors for the channel obtained from the blank.

The ELISA Processor II can store up to 30 different programs for ELISA tests. Programs 1-10 are reserved by the manufacturer for Enzygnost tests and should not be altered, but programs 11-30 are freely programmable by the user according to his needs.

The Behring Nephelometer-Analyzer, depicted in Fig. 14.15, consists of a dilutor, rack station, transfer arm, washing station for the dispensing tip and the cuvettes, cuvette rotor, buffer station, vacuum pump and tank, screen, keyboard, bar code reader and printer.

The dilutor consists of two Hamilton syringes (2.5 mL and 25 µL) used to dose the solvent and the sample, respectively.

The rack station consists of four elements, corresponding to standard sera, samples, diluent and antisera. A motor moves the antiserum rack to the right of the rack station. The other racks are moved jointly by means of another motor. The control sera are introduced into the samples. The sample vessels are bar-coded in order to avoid mismatching. All movements are checked after execution by means of optical sensors.

The transfer arm is furnished with a steel dispensing tip and a microstirrer. The dispensing tip allows samples and standard sera to be diluted and pipetted into the measuring cuvettes. The arm is moved by two step motors in the vertical and horizontal directions. The dispensing tip, also serving as a liquid level sensor, and the microstirrer are washed internally and externally between pipettings.

The cuvette rotor receives the reagent preparations of the samples, rotates the cuvettes for light measurements and places them under the washing station. The rotor is furnished with 45 semi-micro cuvettes —the odd number allows for simultaneous filling, measurement and aspiration. Only every second cuvette is filled. Every 8 s, each cuvette returns to its initial position after two turns. The minimum analysis time is therefore 6 min or a multiple of this.

The washing station consists of four reservoirs holding diluent, buffer-reagent, glycine buffer and washing solution. The reservoirs are fitted with steel tubes provided with liquid level sensors.

The screen, keyboard, bar-code reader and printer link the user with the

instrument. Thus, after introducing the data corresponding to each sample [identification number and parameter(s) to be determined], the instrument gives the position of each sample, reagent and standard in their corresponding rack, performs the required dilutions and mixes the sample and standard with the appropriate reagent, introducing them into a cuvette. The blank reading is taken 8 s after mixing and the final measurement is made after 6 min (fixed-time method) or 30 min (equilibrium method). The values of the different parameters are displayed on the screen and also delivered through the printer if requested. The instrument is equipped with two memory devices: a Winchester hard disk and a floppy disk drive.

The antiserum rack admits a maximum of fourteen flasks (seven in the case of protein determinations as they require complementary reagents). Before starting a protein assay, the user must compile a work sheet with the proteins to be analysed for. If the number of parameters to be determined exceeds the analyser's capacity, it deals with the first fourteen and then requests the introduction of the remaining flasks via the screen. In this way, the analyser can determine up to 70 different proteins; 32 of such determinations are stored in a program and the remainder can be programmed by the user.

Batch analysers using packed reagents fill the gap left by automated analysers, most of which are of little use for carrying out a variety of assays on a few samples in emergencies —they are conceived for the use of reagents prepared daily in large amounts and intended to be added to a host of samples. Generally, there is a starting sequence in which each reagent must be pumped through the system until stabilization is attained; standards and references are used with each batch of samples, or each individually assayed sample, so that a single urgent sample requires the same complete sequence followed for each batch involving a different assay, channel or even development time. This inadequacy for emergency cases is also shared by manual assays, particularly when these are complex and laborious.

The best solution to this problem of analysing isolated samples is to have measured reagents packed in separate units prepared to be used in one-off assays of a single sample. The 'package' also acts as a cuvette for detection. These systems should ideally be stable indefinitely with time and preserve their degree of packing for a given reagent. The two alternatives most widely used in this respect are disposable reagents and dry reagent films.

Du Pont market a series of automated analysers (ACA I, II and III) using disposable pre-packed dry reagents. Each method and position within the system are identified by means of codes. Once the assay (reagent packet) has been selected and the sample has been positioned in the cup labelled with the patient requiring the particular analysis, the instrument performs the sequence of

operations involving selection of the buffer and introduction into the reagent packet, heating at the required temperature, mixing with the sample, incubation, new mixing and measurement (end-point or kinetic). End-point methods use two wavelengths or two packets (sample and blank), while kinetic methods involve two measurements made at 17-s intervals. Absorbances are converted to concentration units by previously stored intercept and slope factors for linear methods or by exponential functions for non-linear immunoassays. Finally, the packet is discharged from the transport chain. The overall analysis time is less than 7 min.

Checking the quality of the analytical packets and developing other assays are the user's reponsibility. Du Pont have marketed an ESI accessory directly linked to the microprocessor via an interface and requiring no special stage for operation. This unit allows the determination of Na and K and is prepared for application of six methods [34].

The use of straightforward reagents on dry films resembling those for photographic purposes was first reported by Gurme *et al.* [34]. The reagent strip typically used is shown in Fig. 14.16. The strip consists of a cellulose matrix impregnated with the reagents required for a given clinical determination. After impregnation, the matrix is dried and stuck by a special adhesive coating to a plastic support, allowing its ready insertion in the measuring system. The matrices can be impregnated with the reagents needed for a series of coupled reactions, so that the chemical interactions involved do not take place until the reagents have been hydrated. Thus, the reagent film for determination of triglycerides contains reagents for four coupled enzymatic reactions. The principle behind these systems is the use of methods involving well-known reactions. The films are normally stable for over 1 year at room temperature —even those containing NADH, which is unstable in wet media. As shown in Fig. 14.16, the film comprises several layers. On the outermost, diffusion layer is deposited a 10-$\mu$L drop of the previously aspirated sample. After incubation at 37°C, the colour development is proportional to the analyte concentration in the sample. It should be emphasized that the even passage of the fluid to the reagent layer through the diffusion layer results in the formation of an area in the centre of the film whose concentration faithfully reflects the analyte concentration in the sample, in spite of the variation in the sample volume —a 10% change in the sample volume results in only a 1% change in the concentration measured for the same sample. The colour is generally measured by densitometry, and the reflectance measured is non-linearly related to the concentration. This technology has been frequently applied to clinical analysis: various methods have been reported for the determination of glucose [35], urea [36], amylase [37], bilirubin [38], triglycerides [39]

and cholesterol [40], and for the simultaneous determination of overall and direct bilirubin [41].

**TABLE 14.2**

Possible uses of urine test strips on other body fluids

| Body fluid | Parameter | Type of result |
|------------|-----------|----------------|
| Spinal fluid | Glucose | Semi-quantitative |
| | Protein | Roughly semi-quantitative |
| | Blood/Hb | Roughly semi-quantitative |
| | Bilirubin | Qualitative |
| | Leucocytes | Qualitative |
| Serum/plasma | Glucose | Semi-quantitative |
| | Bilirubin | Semi-quantitative |
| | Ketones | Roughly semi-quantitative |
| Tears | Glucose | Roughly semi-quantitative |
| | Ketones | Roughly semi-quantitative |
| Fistular discharge | Blood/Hb | Roughly semi-quantitative |
| Drainage fluids | Protein | Roughly semi-quantitative |
| Aspirates | Amylase | Qualitative |
| Dialysates | Bilirubin | Qualitative |
| | Urobilinogen | Qualitative |
| Diluted faeces | Blood | Roughly semi-quantitative |
| | Bilirubin | Qualitative |
| | Urobilinogen | Qualitative |

The measurement of a given biological parameter in different biological fluids normally calls for various test strips. Attempts to use urine test strips for the analysis of other biological fluids, particularly in emergencies, gave unequal results, the most representative of which are listed in Table 14.2. Boehringer Mannheim strips for the analysis of whole blood (Reflotron R analyser) feature two distinct zones: one with a filter for application of the sample drop and another for measurement, towards which the sample diffuses upon passing through the filter.

Cellulose Matrix Containing
Dried Reagents

Plastic Support

Adhesive Layer

Plastic Support
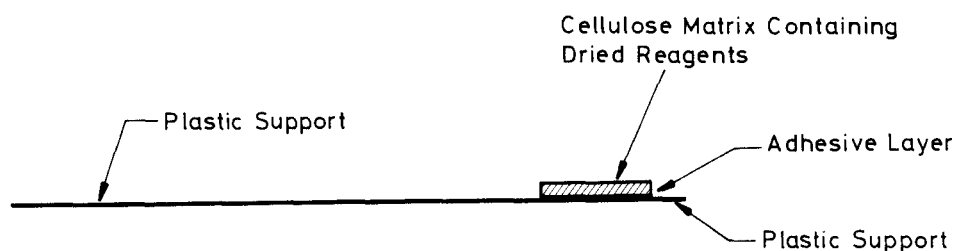
**Fig. 14.16** Solid-phase reagent strip. (Reproduced from [43] with permission of Taylor & Francis).

Figure 14.17 illustrates the foundation of reflectance measurements carried out with this methodology. Figure 14.17b shows the simpler manner of carrying out reflectance measurements. The sample surface is illuminated at a random angle and reflectance is measured at another, also random, angle. This procedure is inappropriate in practice, so it is normally replaced by the use of an integrating sphere (Fig. 14.17a) with a highly reflecting inner surface in which the sample is placed. The light falling on the sample is not directly reflected to the detector; it experiences many reflections until eventually reaching the detector or is absorbed by the wall of the sphere. The reflected light intensity is proportional to that of the colour formed on the strip surface. The complete scheme of the optical system of an instrument of this type is depicted in Fig. 14.18a. The source of the light reaching the integrating sphere is a xenon-discharge tube producing a high-intensity discharge which generates radiation containing wavelengths throughout the spectrum, but largely 340 nm. The strip containing the sample is placed on a thermostated plate which in turn is positioned inside the sphere, also temperature-controlled. The sphere also houses a collimator collecting light reflected by the plate and leading it to an interference filter of the solid-state detector, as well as the entrance to the reference detector, which directs the light reflected by the wall of the sphere to the detector. The net reflectance is the difference between the sample and reference signals, and its use avoids reading errors arising from changes in the light intensity from discharge to discharge. The saphire window covering the outlets of the reference and sample detectors minimize evaporation from the plate surface. The interference filter is specific to each serum assay. The location of the detection system within the instrument is illustrated in Fig. 14.18b, which includes a microprocessor that

acquires and processes the reflectance signals and allows programming of the number of readings to be made on each plate and the interval between measurements [43].
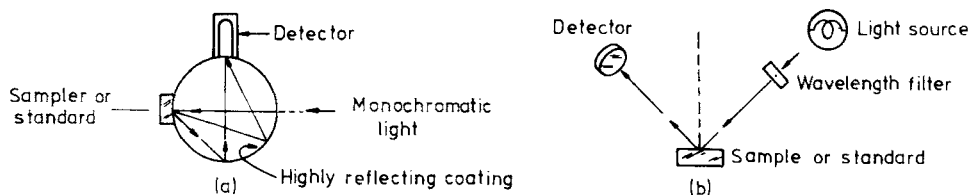


**Fig. 14.17** Basic instrumentation for reflectance measurements. (a) Integrating sphere; (b) simple reflection.

The potentiometric technique has also been used with film methods [42–46]. A potentiometric film consists of a pair of electrodes in the form of thin films of Ag/AgCl deposited on a polyester base and covered with a hydrophilic polymer matrix containing salts; the entire system is covered with an ion-selective membrane. A concentration cell is established in each film by means of two identical electrodes connected via a porous paper bridge, so that a potential difference across the cell is generated by placing a drop of sample on one electrode and another of blank on the other. Phosphorescence [47], fluorescence [48], IR spectrometry [49] and photometry with optical fibres [52] have also been used, although less frequently, as detection techniques in monitoring the development of reactions taking place on dry films.

Kodak recently developed an instrument for implementation of the dry film technology. The so-called Ektachem 40 is a microprocessor-controlled device capable of performing up to twelve assays simultaneously at a rate of 300–500 analyses per hour. Spectrophotometric analyses require about 6 min, and potentiometric assays only 4 min.

A typical example of fluorimetric determination on dry reagent films is that of serum immunoglobulins reported by Wang *et al.* [48]. The method uses a stabilized solid-phase immunoadsorbent consisting of an antigen immobilized on a cellulose nitrate–acetate disc stuck to a Stiq plastic sampler. The polymer disc acts both as a substrate for immobilization and as an even surface on which highly precise fluorimetric determinations can be conducted. The assay

involves the reaction of an accurate, limited amount of a specific fluorescent antibody with the specific antigent present in the prepared sample. The fluorescence of the bound labelled antibody is inversely proportional to the amount of antigen present in the measured sample and is measured with a FIAX solid fluorimeter. The sequence of events involved in the determination is illustrated in Fig. 14.19. The culture tube contains the sample and the fluorescent antibody and the second tube holds the buffer. The fluorescent signal yielded is logarithmically related to the antigen concentration in the sample. The instrument is capable of measuring about 60 Stiq samplers in 5 min.
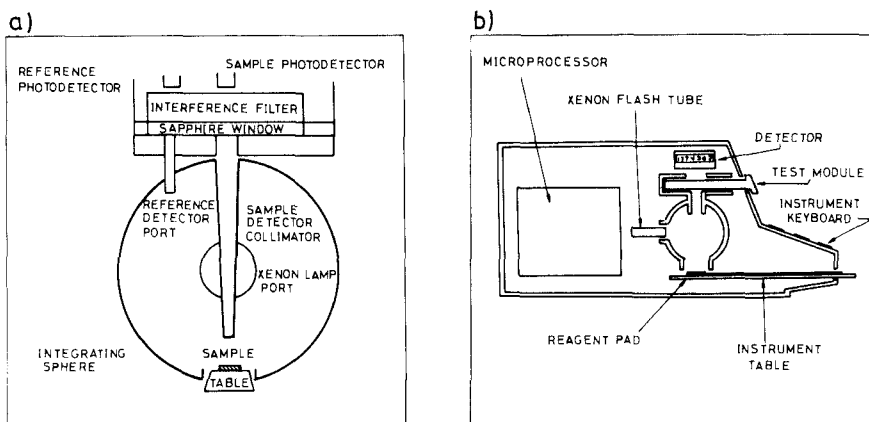


**Fig. 14.18** (a) Complex optical system for reflectance measurements. (b) Location of the detection system. (Reproduced from [43] with permission of Taylor & Francis).

## 14.4 IN VIVO MEASUREMENTS

In contrast to the subjects of other fields, biological systems always run the risk of being physiologically unbalanced as a result of sampling. The consequence of this disturbance is that, however reliable the analysis may be, one never measures the actual value of the parameter of interest. This problem is lessened by performing *in situ* or *in vivo* measurements, which, however, require sterilization and calibration. *In vivo* measurements can be classified according to whether they are made under a static or a dynamic flow.

Static measurements are carried out on the blood stream, the heart-lung circuit or, post-operatively, vein by-passes. These measurements are effected with ISEs in most instances; hence their main problem is their sterilization, as the sensors rarely withstand temperatures above 50-100°C. Other problems related to their use are (a) a lack of knowledge of the toxicicty of the active

phases in the liquid ion exchangers making up the ISE membranes, (b) the deposits of albumin gathering on the surface of glass electrodes, which lengthen measurement times and cause potential derivatizations, and (c) the formation of disturbing coagula.



| | 1 | 2 | 3 |
|---|---|---|---|
| | React | Wash | Read |
| 0:00 | 0:20 | 0:40 | 0:45 |
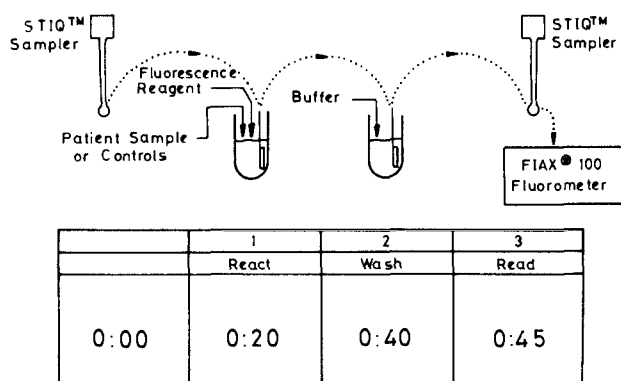
**Fig. 14.19** Steps of the determination of serum proteins by use of a FIAX[TM] instrument.

Home-made ISEs for *in vivo* measurements are usually constructed from micropipettes, the tips of which are broken under a microscope to obtain tips 1–6 µm in diameter whose borders are flanged in the flame to avoid damage to the cells upon contact with the sensor (extracellular mesurements). These small sensing surfaces minimize one of the major sources of error in this measurements, namely sample contamination through loss of saline from the bridge. On the other hand, their small surfaces make their handling rather cumbersome.

Glass capillary electrodes for pH measurements are probably the commonest of all commercially available sensors of this type. That manufactured by Radiometer is thermostated and allows the measurement of the pH in 25 µL of capillary blood with a standard deviation of 0.006 pH unit. Although there are various sources of uncertainty common to the different ways of determining ionic activities, those involved in pH measurements are more serious. This is a result of the most important biological buffer containing a gas, $CO_2$. *In vivo* pH measurements have the advantage of eliminating the uncertainty arising from the random selection of a $CO_2$ pressure with which to balance the samples. This, in addition, avoids losses of $CO_2$ to the atmosphere.

The instrument marketed by Radiometer for transcutaneous measurements of $pCO_2$ and $pO_2$ consists of two separate portable units readily furnished with a

recorder. The sensing system has two parts: the electrode body, linked to the connection lead, and a disposable, flexible attaching ring for easy and quick adhesion to the skin. The liquid contact (a non-toxic electrolyte causing no damage to the skin) is applied after attaching the ring, which is applied dry. The ring should be periodically replaced in long-term monitoring.
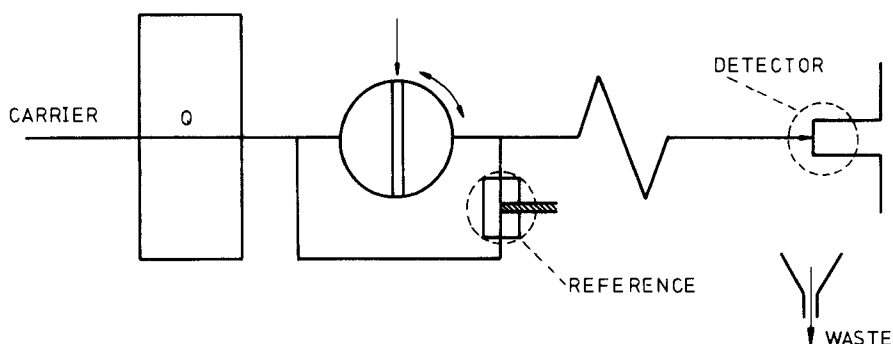


**Fig. 14.20** FIA system for *in vivo* measurements.

The FIA-ISFET association is an alternative of great potential to this type of measurement, but hardly exploited to date. The advent of ISFETs (ion-sensitive field-effect transistors) [52] has resulted in considerable reductions in the size of potentiometric detectors based on this principle, to the point of being used in hypodermic syringes or probes for *in vivo* measurements. The influence of the flow (rate and angle of impingement), adsorption and viscosity on the membrane can be determined with the aid of an FIA system such as that depicted in Fig. 14.20, in which the detector is continuously exposed to a flowing stream. These experimental conditions mimic *in vivo* measurements, where the ISFET is placed in the blood stream. The problems derived from the flow-rate can be minimized by selecting a suitable electrode angle. The chief shortcoming of the use of ISFETs for *in vivo* measurements is the adsorption of red cells by the selective membrane and the consequent clogging of its surface and gradual increase in the signal generated. This problem could not be avoided in *in vivo* measurements and has long been a common problem with the polymer membranes used for potentiometric detection. The combination of FIA and ISFETs avoids this drawback by simply using a buffer or electrolyte as carrier and connecting the system's injection to the blood stream via a suitable tube, so that blood circulates continuously through the sample inlet and is sampled at regular intervals (e.g. 1 min) by simply switching the sample

valve. In this manner, the detector, and hence its membrane, is only exposed to the injected portion of blood (between 3 and 30 μL), and for only 10 s, while the buffer solution is exposed for 50 s prior to injection of the following sample, thereby washing off the red cells from the membrane. The problem of the electrode calibration is also solved by connecting the channel carrying the calibration solution to the system, the sensor being calibrated at preset intervals by alternating its injection with that of the blood sample, thereby ensuring maximum precision [53]. The only problem posed by this FIA system is the need for the insertion of a small volume of carrier into the bloodstream when the injection valve is switched to its filling position. This problem can be readily overcome through a suitable modification of the injection system.
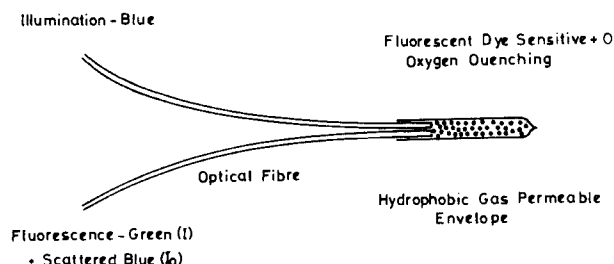


**Fig. 14.21** Fluorimetric probe for *in vivo* measurements of pO$_2$ based on dry chemistry. (Reproduced from [54] with permission of the American Chemical Society).

A new type of probe for *in vivo* measurements based on the used of solid reagents and fluorimetric detection has been reported [54]. The instrument affords pO$_2$ measurements and is based on the fluorescence quenching principle. It consists of two optical fibre leads of 250 μm ending in a tubular section of porous polymer about 3 mm in length and 0.6 mm in diameter. The tube is filled with a dye absorbed on a support. It is similar to the pH electrode used with dry reagents, but requires solving three major problems encountered, namely: (a) it requires a dye whose measured property, fluorescence in this instance, is quenched by oxygen, which is excited by visible light and is resistant to the loss of the aforesaid property; (b) it requires the use of a hydrophobic wrapping that is highly permable to oxygen; (c) it needs an adsorptive support activating the dye but insensitive to moisture. The probe is de-

picted in Fig. 14.21. The dye supported on the adsorbent is contained in a porous propylene tube allowing rapid equilibration with the surrounding oxygen, thus avoiding contamination of the dye. The blue exciting radiation is passed through an optical fibre to excite the dye. The fluorescent radiation, green, is driven alongside the dispersed blue light to the measuring instrument via another optical fibre.

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# 15

## Automation in environmental pollution monitoring

### 15.1 INTRODUCTION

The environmental deterioration caused by human action in the last few decades is alarming as demonstrated by the large number of laws aimed at environmental protection passed in recent years, so much so that legal historians have come to call the 1970s "the great era of environmental law". Among others, these laws establish the need for extensive measurements intended to detect the presence and concentration of a large variety of compounds, both organic and inorganic, in a pleiad of different samples. The major thrust behind the passage of new legislation has been the correlation of adverse health or ecological effects with measurements of the presence and concentration of a variety of pollutants. Such measurements are possible today thanks to significant advances in analytical instrumentation and chemistry, electronics and computer science in the last two decades. However, there is still a wide gap between the development of sophisticated automatic methods of analysis and their application to routine measurements of environmental pollutants [1]. This is no doubt the result of the high cost of automation and the lack of awareness of the real potential and limitations of these sophisticated methods.

The large variety of pollutants that may in principle occur together in a given matrix, whether gas, liquid or solid, poses problems requiring specific solutions based on particular methods. The selection of a method for the solution of a given problem usually has two goals. The so-called 'target compounds' (TCs), whose concentrations are desired are usually contained on a list submitted to the analytical laboratory. On the basis of this list, the laboratory optimizes sampling and the measurement method to be used. Detailed chemical instrumental procedures are designed to isolate the target compounds from the sample matrix and known interferences, concentrate and measure them with various all-purpose or selective detectors. Laboratory control standards are usually employed to establish that isolation does occur and to optimize the procedure for maximum recovery of the desired components and minimum interference from other components. The gas chromatographic procedures with electron-capture detector for chlorinated pesticides are examples of the

optimized TC approach. However, the justification for the selection of a specific method requires more information than the decision to measure target compounds.

The broad spectrum (BS) approach is in sharp contrast to the TC approach. The idea behind the former is to seek a broad spectrum picture of whatever is present in a sample as a major or minor component. This kind of analysis is not guided by a predetermined list of compounds to be measured. The BS approach has existed ever since the beginning of chemical analysis. However, the practical and economic pursuit of this goal was often not possible in the past. The development of computerized gas chromatography/mass spectrometry systems and other similar technologies has made this goal a feasible and desirable alternative for many types of samples. With the BS approach, sample preparation is designed to be as simple as possible to preclude losses of significant sample components and to minimize the possibility of sample contamination. The idea is to divide the sample into broad classes of compounds and to apply all-purpose chromatographic methods for the separation of the compounds in each group. Literally hundreds of thousands of compounds can be potentially included in a broad class, but it is usually safe to assume that only a few compounds are present in each class in most samples. The most beneficial result of the BS approach is the frequent discovery of significant but previously unrecognized pollutants.

If the decision is made to use the BS approach, method selection is restricted. Gas chromatography/mass spectrometry may be a viable choice. On the other hand, if the decision is made to use the TC approach, careful consideration of the sample type is needed before a method is selected.

Environmental systems can be divided into three broad categories:

*Type 1* systems are relatively closed, i.e. there is some control of the entry of components into the system and all components are well defined. An example is the output from a chemical plant that uses raw materials of know composition, processes them according to a particular procedure and generates well-defined products and by-products.

*Type 2* systems are somewhat open in that entry of new components is possible but not frequent or likely, and components are tentatively defined. An example is the output from a drinking water treatment plant tha uses an uncontaminated ground water source and chlorinates it to produce a more or less constant variety of halogenated methanes.

*Type 3* systems are wide open to entry of almost anything at any time and components are poorly defined. An example is the Mississippi River at St Louis, MO.

Table 15.1 lists the principal species and parameters controlled in the en-

vironmental field, as well as the techniques used for their detection and quantitation, described in detail in the different sections of this chapter.

**TABLE 15.1**

Species and parameters most frequently determined and techniques most commonly used in environmental analysis

| Species/parameter | Technique |
|---|---|
| *Metals:* | |
| Hg, Pb, Cd, Ca, Mg | Flame and cold-vapour atomic absorption. ICP |
| Fe, Mn, Al | atomic emission spectrometry. Graphite furnace. |
| | Zeeman effect. Anodic stripping voltammetry. |
| | X-ray fluorescence. ISEs. Colorimetry |
| | |
| *Anions:* | |
| $NO_3^-$, $NO_2^-$, $PO_4^{3-}$, | Colorimetry. ISEs. Laser Raman spectroscopy. |
| $SiO_3^{2-}$, $CN^-$ | |
| | |
| *Oxides/Acids:* | |
| $SO_2$, $NO_x$, $H_2S$ | Fluorimetry. Coulometry. Colorimetry. LIDAR. |
| | |
| *Other compounds:* | |
| $NH_3$, $NH_2NH_2$, $O_2$, $Cl_2$ | Colorimetry. Chemiluminescence. ISEs. |
| | |
| *Oxygen demand:* | |
| BOD, TOD, COD | ICP-AES. Colorimetry. |
| | |
| *Organic compounds:* | |
| Pesticides, PCBs[1], | Gas chromatography. HPLC. |
| PAHs[2], detergents, | |
| sludge digesters, | |
| $CO_2$, $CH_4$ | |
| | |
| *Other parameters:* | |
| pH, temperature, | Potentiometry. Digital thermometer. Thermistor. |
| turbidity, conduc- | Thermocouple. Turbidimetry. Nephelometry. Con- |
| tivity | ductimetry. |

[1] Polychlorinated biphenyls     [2] Polyaromatic hydrocarbons

The availability and preparation of standards is a major problem in the environmental field. There are standards for many relatively stable constituents such as metals, nutrients or parameters such as turbidity or alkalinity to name but a few. However, there is a demand for standards for dissolved oxygen, suspended solids, chlorine, biochemical oxygen demand, oils and fats. It would also be a great aid to have pH standards containing some of the organic substances commonly present in water and introducing errors in the determinations.

The analysis of samples at the trace or ultratrace level poses special problems. As a rule, dilute standard solutions are unstable and readily contaminated. It is therefore essential to have a variety of independent, accurate standards over a wide range of trace concentrations. At present, the National Bureau of Standards has only a few Standard Reference Materials (SRMs) suitable for the analysis of plant, animal and soil materials, none of which is usable in the trace or ultratrace range.

The analysis for metal ultratraces requires extremely clean laboratories [2]. This, in turn, requires the use of a carefully filtered air supply. All reagents used must be certifiedly ultrapure and their purity be preserved. Deionized water should be carefully controlled to ascertain the absence of contaminating traces —this requires all technicians to exercise care to avoid contamination by their hair, skin, cosmetics, excretions or even the exhaled air. A strict control of the analytical blank [3] is obviously another must in this type of analysis to avoid spurious results in the determination of some trace or ultratrace analytes.

## 15.2 SAMPLING

### 15.2.1 General considerations

Sample collection —and transport and storage— represents one of the chief problems with which the environmental analyst is confronted. The inherent difficulties posed by the analysis of some types of materials are augmented by the specific problems presented by the environmental field. The sampling operation may disturb the target system to the point of obtaining a final sample irrepresentative of the unaltered system. This may not be too much of a problem with atmospheric measurements, but it can pose serious difficulties with biospheric samples. Thus, collection of an air sample for analysis is not bound to cause much disturbance to the surrounding air; however, the measurement of the glucogen level in a rat's liver will inevitably result in a great disturbance to the animal's organism. These examples are illustrative of one of the major features of environmental samples: their heterogene-

ity. The glucogen measurement is truly representative insofar as the entire organ is exposed. Conversely, atmospheric measurements are hardly representative of even a limited volume inasmuch as the homogeneity of the sampling field depends on the stratification in the vertical plane and, to a lesser extent, on the possible variations in the composition in the horizontal plane. This entails carrying out multiple samplings to ensure reliable and representative measurements.

Time is another key variable in environmental sampling, not only as regards the sampling frequency, which should be high enough to show any changes in the sampled material (e.g. tidal variations in estuary water), but also as regards the interval elapsed between sample collection and analysis, which should be short enough to ensure that no essential information is lost through the sample deterioration. Time is also a crucial factor when the results of the analysis are the basis for decisions on the environment —particularly the working environment. The accessibility of the sampling spot is also highly influencial in the procedure to be employed in collecting samples [4,5].

The pleiad of problems associated with sampling have led to the development of specific instrumentation for environmental analysis avoiding this preliminary step (e.g. chemical sensors capable of performing *in situ* measurements). The most significant developments in this respect have been aimed at the area posing the greatest problems in sampling, namely the biosphere. In fact, *in vivo* measurements of some parameters such as alkali-metal and alkaline-earth activities, pH, dissolved oxygen, etc. are by now affordable [6–8].

### 15.2.2 Sample storage

Unfortunately, not many substances remain unaltered after sampling. The chief causes of deterioration of collected samples are losses of trace elements by adsorption, particle segregation in heterogeneous powders, dehydration, bacterial growth in biological samples, decomposition of the sample matrix and formation of volatile compounds from trace elements. Most of these problems can be addressed with specific techniques which, however, may introduce some contamination. The potential occurrence of changes in the sample during storage is closely related to the storage temperature and time. Plastics —particularly PTFE and polyethylene [9,10]— are the best material for storage of the collected samples, which can also be preserved by cryostorage. This reduces the sample activity to such an extent that deterioration and interaction tact with the container are kept to a minimum. Acidification is a common resource for sample stabilization; however, it may be contra-indicated in speciation studies [11]. The absorption tubes used in air sampling are suitable for storage of gas samples as described in Section 15.2.5.

### 15.2.3 Sample clean-up

The nature of environmental samples often requires a clean-up step between sampling and the application of the analytical method. There are a variety of clean-up procedures available, most of which can be implemented in an automated fashion (see Chapters 3 and 4), in both segmented and unsegmented flow-methods, robotic methods and HPLC. Liquid-liquid and solid-liquid extraction [12-14], filtration [14,15], dialysis [12,15], evaporation [12,13], low-temperature precipitation of lipids and column-switching methods [16] are all clean-up methodologies of proven efficiency with environmental samples.

### 15.2.4 Liquid sampling

Water and liquid effluent samples possess the major disadvantage of appearing to be very easy to obtain compared with solid or gaseous materials: a flask tied to the end of a string and a bottle to collect the sample seem to be all the equipment required. For comparison, a factory processing 100 t of coal a day will have to devote much endeavour to organize its appropriate sampling; a faecal water-treatment plant dealing with 20 000 t of material daily would require an individual inspecting the input and output of material at certain intervals by using a small flask to collect samples from each stream and pour them into a larger container from which samples would be analysed at the end of the day. Alternatively, a river inspector would make a monthly inspection along the river, collecting samples that would also be analysed at the end of the day. The results obtained would provide a pollution contour of the river in question. Taking into account, for instance, that the flow-rate of the Thames at Teddington weir is stated to be 277 200 t/h and that of the Trent at Colwick is 2 160 000 t/h [17], there is a great difficulty in relating sampling to a river flow-rate. From these examples it follows that (a) the significance of the analysis is limited by the suitability of the sampling program and (b) the chief requirement for a satisfactory sampling is the representativeness of the collected samples.

The automatic sampling of liquids can be carried out with off-line and on-line samplers. Off-line assemblies consist essentially of a suction pump, a series of flasks and microprocessor allowing programming of the intervals between successive samplings. An example of this type of set-up is the portable automatic sampler S-4000 of the Philips Environmental Protection Series, a scheme of which is depicted in Fig. 15.1a. The operational sequence involved the following events:

(a) The sampling cycle control (1) initiates the sequence by means of an internal quartz-crystal-controller timer with accuracy of better than 0.03% or an external signal. Cycle intervals can be set between 3.75 min and 24 h. The

compressor (2) pressurizes the measuring chamber (4) and purges the intake hose (7).
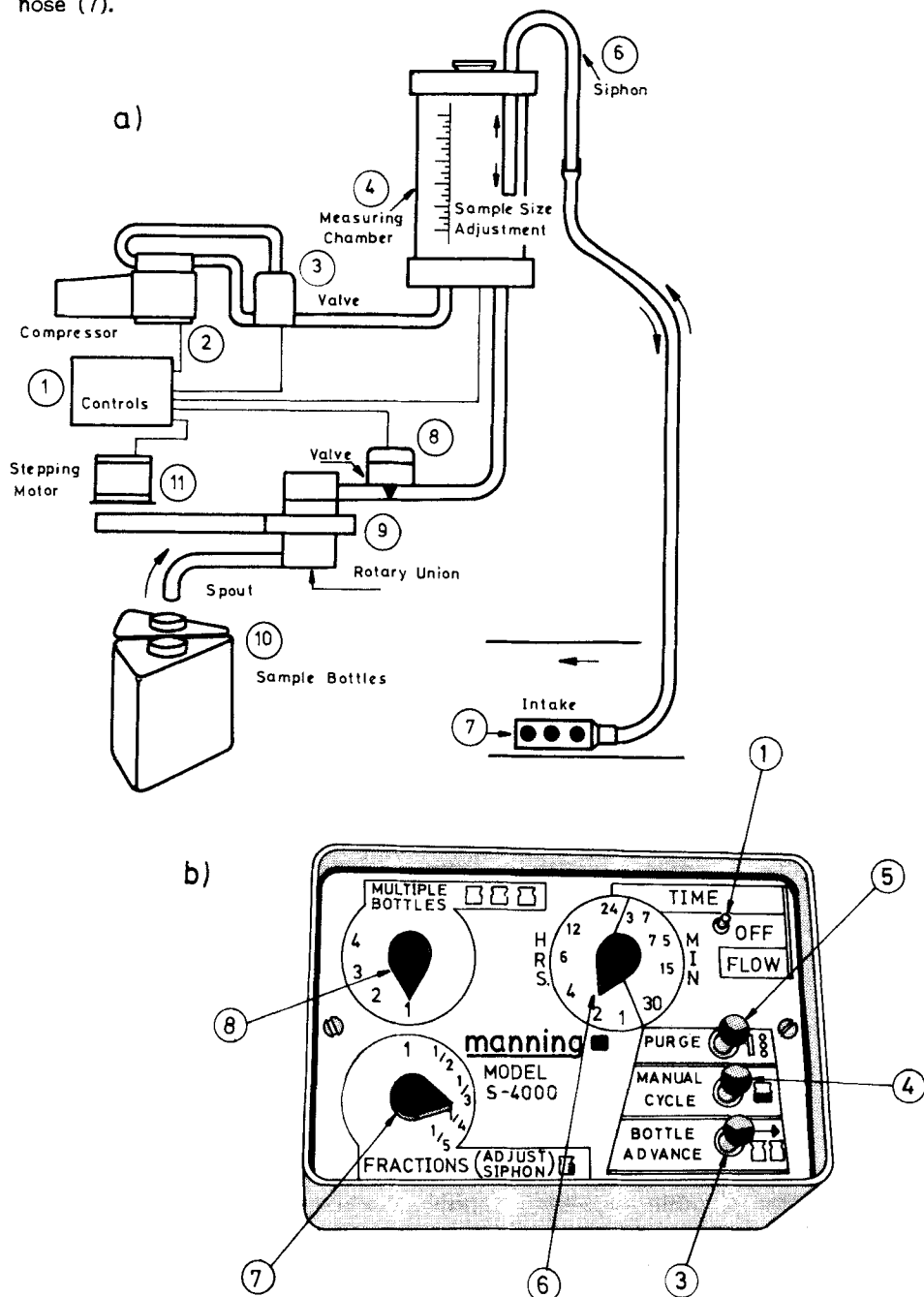


**Fig. 15.1** Portable Automatic Sampler S-4000. (a) Operational diagram. (b) Control system. (Courtesy of Philips).

(b) The solenoid valve (3) inverts the compressor lines to evacuate the metering chamber (4). The sample is aspirated from (4) into the chamber.

(c) Once the sample has been collected, pinch valve (8) compels the sample to circulate through the rotary union (9) and pours it into the sample bottle (10).

(d) Pinch valve (2) is closed and the purge of the intake hose is continued.

(e) The compressor shuts off, steeping motor (11) advances to spout to the next bottle and the system is made ready for the next cycle.

The control unit (Fig. 15.1b) is located at the top of the system. The mode switch (1) selects the type of control of the cycle. In the 'flow' position, the sampling cycle and the timer are controlled by the quartz-crystal clock, intervals being selected through the sample interval switch (6). The sample volume is selected by adjusting the height of the sample used to introduce the sample into the metering chamber, scaled in volume units. The bottle advance push-button (3) moves the bottle forward and the manual cycle (4) push-button (4) initiates one complete cycle to test the unit. The purge push-button starts the purge cycle and stops it when released. The optional controls for multiple samples (7) and multiple bottles (8) allow 1-5 samples to be placed in a single bottle and up to four consecutive bottles to be filled with the same sample, respectively.

On-line sampling systems are simpler than their off-line counterparts and normally consist of a floating piston housed in a cylinder, generally plastic, which provides a flow of adjustable rate. Such a rate should not be too low in order to avoid sedimentation. The plastic cylinder is used in variable lengths and diameters and is pierced and surrounded by a mesh preventing the entry of large particles that might obstruct the pump. The cylinder is thermally isolated to avoid solar radiation or temperature variations that might result in erroneous measurements.

Whitby *et al.* [18] have designed a sampler that can be used with on-line and off-line systems. It was conceived for the sampling of effluents whose composition varies with time or which contain solids heavier or lighter than the fluid itself, volatile solutes or other immiscible liquids. The design is based on the use of a loop of fairly narrow-bore pipework, which is fed from the body of water to be sampled. A mono pump is used to force the sample continuously through the loop (Fig. 15.2a) at a rate sufficiently high (500 gal/h) to promote turbulent flow and also to prevent the settlement of high-density solids by maintaining a high linear flow velocity. This stream of liquid then passes downward through a small closed interceptor chamber which has a limited retention volume (2-3 L) and the liquor then flows under gravity
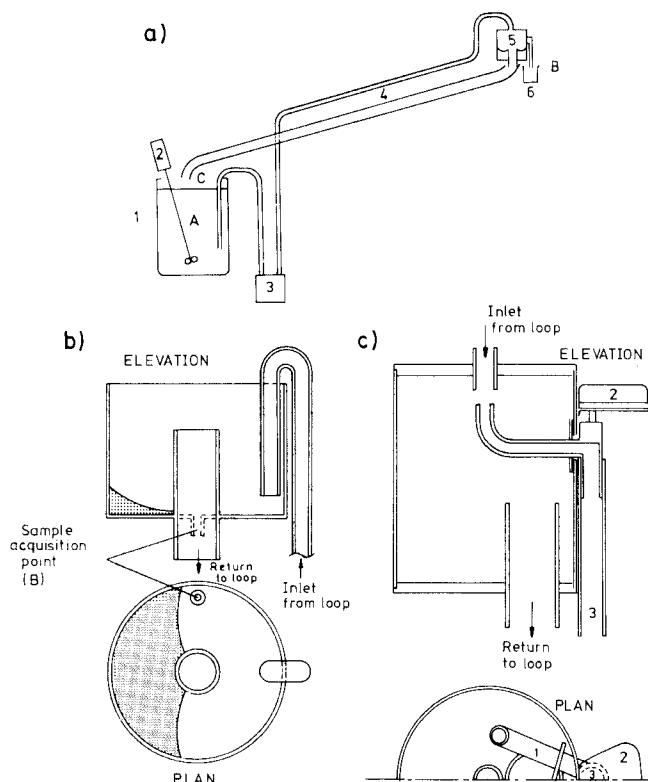
**Fig. 15.2** (a) General layout of the loop-interceptor system for stud-
ies on a simulated effluent. (1) Thirty-gallon drum holding the ef-
fluent; (2) lignin mixer; (3) mono pump; (4) outward and return lines
of loop; (5) interceptor; (6) sample point. In the real situations,
points A and C are located in the flow of liquid effluent. (b) Inter-
ceptor vessel (early design). Shaded areas indicate the area in which
suspended solids build up. (c) Final design of the interceptor vessel.
(1) Swivelling sample-acquisition arm; (2) Kinetrol actuator; (3)
sample delivery tube —represents sample point B. (Reproduced from
[18] with permission of Pergamon Press Ltd).

and is discharged near the point from which the original sample was drawn.
This assembly can be used to promote vigorous agitation at the initial sample
point, so that the liquid drawn by the pump is representative of the —pos-
sibly heterogeneous— effluent stream. The interceptor vessel can be placed at
a position that is convenient for the collection of sub-samples and may also
be used to house pH or ion-selective electrodes to give a continuous record of
the chemical characteristics of the effluent. The interceptor provides a means

for diverting a sample from the loop system into a suitable sample container. However, by suitable electronic or pneumatic timing arrangements, the pneumatically operated sampling arm may be arranged to automatically collect samples of the following types: (a) snap or grab samples at chosen time intervals; (b) composite samples; (c) flow-proportional samples; (d) continuous samples. The arrangement of the loop system is shown in Fig. 15.2a. The outward loop feeds the interceptor vessel from which samples may be withdrawn (Fig. 15.2b) or which, in improved form (Fig. 15.2c), permits removal of sample and also insertion of monitoring probes into the continuously updated sample. The sample loop is completed via a pipe which returns the sample close to the pump intake point, thus providing turbulence under real operating conditions.

The instruments with on-line sampling units used in surveillance stations can also include a programmed system for emergency samplings, allowing samples to be collected as soon as any of the controlled parameters exceeds a preset limit. This allows samples to be obtained in cases of transient or unexpected pollution.

### 15.2.5 Air sampling

Most of the sampling carried out in the atmosphere is intended for the determination of pollutants or their effects in localized industrial areas or areas devoted to global environmental studies. Only ocassionally (e.g. measurements of boron [19] or nitrogen oxides in air [20]) are these studies aimed at the identification of the geochemical cycle of some species in the atmosphere.

Systems for sampling of airborne pollutants usually consists of three parts: (a) a means of collecting the air sample; (b) a device to trap the pollutant; and (c) a means of measuring the amount of air sampled. The sampling methods frequently used for this purpose are sedimentation [21,22], centrifugation [23], impaction, filtration and thermal or electrostatic precipitation [24,25], the commonest of which are filtration, impaction and electrostatic precipitation. The size of the collected particles depends on the particular method used.

Filtration is the sampling method most frequently used in industrial hygiene work (personal monitors) on account of its operational simplicity. A filter assembly typically consists of a sampling head and a filter (passive atmospheric samplers) or a sampling head, filter and pump (active samplers), all of which are constructed in a material introducing no contamination in the samples. The different types of filters (depth, membrane) and materials (glass fibre, quartz, cellulose esters, PFTE, silver) used can be readily adapted to the unknown analyte.

The control of working atmospheres requires a special type of sampling. In fact, the level of pollutants to which the individual workers are exposed is more representative than that found in the working place. Obviously, in a petrol station, the individual actually dispensing the fuel will be more exposed to organic vapours than the cashier, even if the latter is only a few metres away from the former. A common method for sampling in these 'microenvironments" requires the workers to carry a badge with adsorptive material —typically a charcoal disc pressed into a filter backing— during the working hours (passive sampling), at the end of which the badge is sent to the laboratory, where carbon is extracted with an organic solvent (generally $CS_2$) and an aliquot is injected into a gas chromatograph for analysis. This simple method has some disadvantages: (a) the uptake rate of the disc when worn will be affected by the air flow across it and it will perform poorly in still air; (b) the most effective solvents are themselves a health and safety hazard; and (c) the many handling and extraction steps involved do not lend themselves to a fully automatic system of analysis.

Perkin-Elmer have developed a sampler that can be used both for the adsorption of samples by diffusion —as a badge sampler— and for the introduction of a controlled air stream by means of a portable battery-operated suction pump, which allows for spot sampling or the measurement of very low pollutant concentrations. The sampler is subsequently heated in a flow of carrier gas and the vapours are fed directly into a gas chromatographic column. The monitor consists of  stainless steel tubes (90 mm x 5.0 mm I.D.) (Fig. 15.3) that can be readily removed with a clip. The tubes are supplied with press-on storage caps and gauzes which retain the sample inside for months if necessary. In the sampling mode, the tube is fitted with a diffusion cap constructed so that the diffusion path length is set precisely to 15 mm. Diffusion caps are available with semipermeable membranes to minimize water uptake during sampling, without affecting component diffusivity. These caps have a gold-anodized finish for easy identification and are also available without membranes for non-moisture sensitive materials —these have a silver cap. The analysis is carried out on the Perkin-Elmer Automatic Thermal Desorption System (ATD 50) and then analytical end caps are fitted. These maintain the sample sealed in the ATD unit and enable the sample to be dispensed to the chromatograph without loss or contamination. The ATD 50 automatically processes up to 50 sample tubes without attention. Complete control of all desorption parameters coupled with an extensive systems check before each tube is desorbed ensures that each sample is subjected to an optimum desorption cycle and cannot be lost due to system or tube failure. The desorbed sample passes along a temperature-controlled transfer line which may be attached to any gas

chromatograph or vapour-phase analytical system. An interesting feature of these tubes is their reusability.
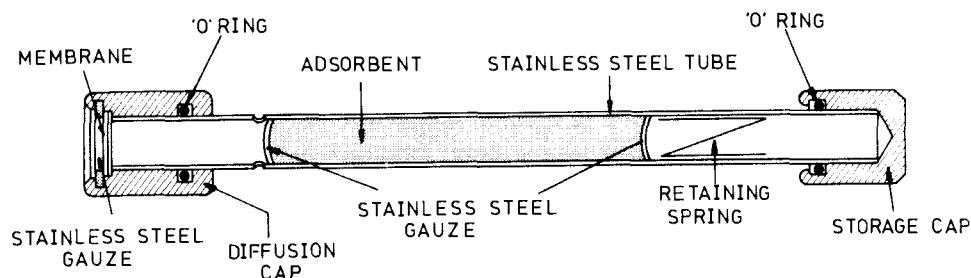


**Fig. 15.3** Re-usable sampling tube (active or passive). Analyses are carried out on an Automatic Thermal Desorption System fitted to a gas chromatograph. (Courtesy of Perkin-Elmer Ltd.).

A pump is usually employed to transport the air sample along the sampling device, where the air stream impinges on a solid surface (impactors or adsorption tubes) or a solution (impingers) where the particles or gases settle. The solid use for impaction or adsorption and the absorbing solution are suited to the type of compound to be determined and to the nature of the sampled atmosphere.

Electrostatic precipitation is specific for suspended particles and is thus widely used in this type of sampling [26,27].

The collection of gas samples, both organic and inorganic, for analytical purposes has been approached by various authors. Thus, Smith and Murdock [30] have developed a device capable of simultaneously collecting eight equivalent particulate diesel exhaust samples to study the effect of the type of filter, extraction solvent and storage conditions used on the measurement of the concentration of five polyhalogenated hydrocarbons. The device consists of a conical sampling chamber (18.5 cm diameter, 15 cm height) on which are placed eight cassettes of two 37-mm pieces each, a vacuum manifold containing the critical orifices which control the flow through the 37-mm cassettes, and a sample line for attaching the conical sampling chamber to the diesel exhaust dilution chamber. A 16.5-mm diameter by 26.5-cm long Plexiglass tube is used to support the sampling chamber and to separate it from the manifold. A threaded rod with wing nuts is used to hold the sampling chamber, vacuum manifold and Plexiglass tube rigidly together. The sampling line, a 2.54-cm

a)



SIDE VIEW

SAMPLE LINE

37mm CASSETTES

THREADED ROD

PLEXIGLAS TUBE

CRITICAL ORIFICES
8
VACUUM MANIFLOD

TO PUMP

b)

37mm CASSETTES (8)

LEFT SIDE

RIGHT SIDE

TOP VIEW(with top off)
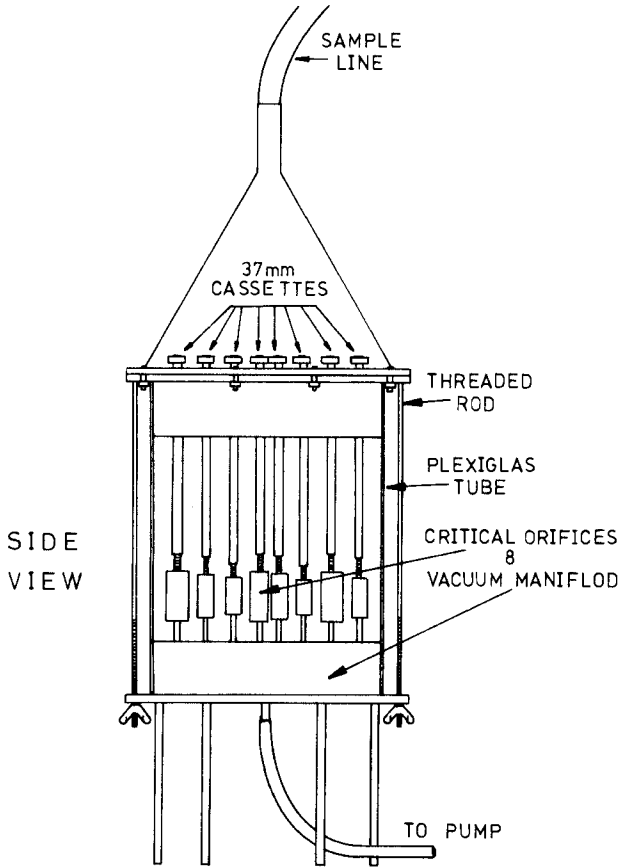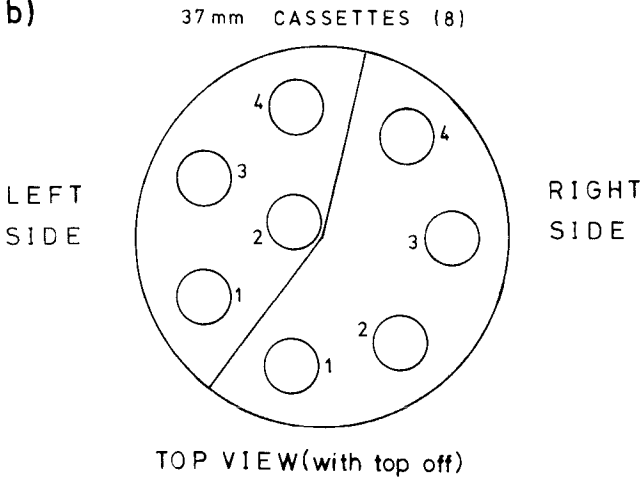
**Fig. 15.4** Multi-sampler for collection of polyhalogenated hydro-carbons in diesel exhaust. (a) Side view. (b) Top view. (Reproduced from [30] with permission of Marcel Dekker, Inc.).

aluminium tube is inserted into the diesel exhaust dilution chamber. The 37-mm cassettes are arranged in the sampling chamber as shown in Fig. 15.4a. The pattern is divided into a right and a left section, and each position is numbered individually (Fig. 15.4b). Similar studies of collection efficiency, blank values and elution efficiency of lead sprays collected over four different solid sorbent packing sampling tubes (silica gel, alumina, Chromosorb and Tenax-GC) in various pore sizes, which showed the suitability of Tenax-GC, have been recently reported [31]. Tenax-GC was also used by Lewis *et al.* [32] to develop a passive sampler for short-term, flow-level air monitoring applications. The small, stainless steel device is straightforward and inexpensive and has a high equivalent sampling rate, is reusable and rechargeable and is conceived for thermal desorption. Its performance was scrutinized under controlled test chamber atmospheres and in actual outdoor and indoor situations. Sampling rates were calculated for several volatile organic chemicals. This design excels in performance over other commercially available passive monitors.

Sample collection as a means of avoiding a given interference and determining other analytes has materialized in the development of a KI ring denuder for the collection of $NO_2$ [33] as a means of separating this oxide prior to the sampling of airborne particulate matter on filter media such as polycyclic aromatic hydrocarbons liable to undergo oxidation or nitration in the filtration step [34,35].

A representative example on account of its capacity and performance is the commercial on-line gas sampler DS 210 dry-air-operated sample-conditioning probe manufactured by Columbia Scientific Industries. This sampler effects in a precise manner all the functions involved in the preparation of the in-stack sample for transport and measurement. The sample is first filtered and metered to an exact volume by a critical orifice. The measured sample is then diluted with dry eduction air, reducing the relative humidity to a dew point below that of the ambient operating temperature. A vacuum is drawn on the stack continuously through a filter and a critical orifice by a newly developed small ejector pump which is mounted inside a stack probe. The main air stream (pressurized air) with an adjustable flow creates a vacuum in the space between the primary and secondary nozzle of the ejector pump (Fig. 15.5). The vacuum is used to transport the stack gas through a critical orifice mounted inside the stack probe. The critical orifice determines the stack sample flow at all pressures below the critical value. Figure 15.5 shows a detail of the construction of the ejector pump, which is mounted inside the stack probe inside a steel tube of highly corrosion-resistant steel which forms part of the stack probe. Between the ejector and this outer steel tube a heat exchanger serves to preheat the dilution air before entering the pump so as to compensate for

changes in the dilution ratio at varying temperatures of the dilution air. The second part of the probe consists of a corrosion-resistant steel mantle which is screwed on to the ejector pump end. On the front end of this mantle is mounted a coarse filter. Inside this·part of the probe is mounted the critical orifice with a fine quartz filter. Both can be exchanged for other sample flows. The CL/PA (calibration/purge air) connection of the probe consists of a 1/8-in steel tube which ends in the front compartment. This tube is used to supply the critical orifice inlet with calibration gas supplied via the umbilical cord or to apply purge air at a high flow-rate to clean the coarse filter. The air stream blowing into the stack removes the dust particles remaining at the outside of the coarse filter. The diluted sample volume is approximately 5 L or more —sufficient to meet the sample needs (simultaneously) for several analytes, such as total sulphur, $SO_2$, $NO_x$, $CO_2$ and hydrocarbons.
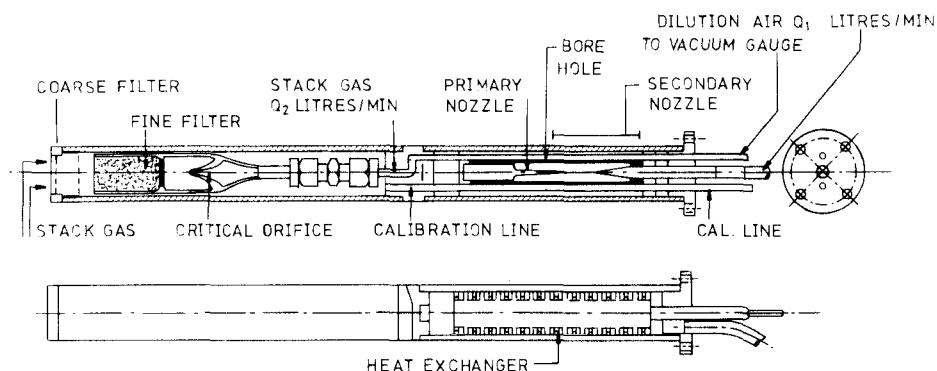


**Fig. 15.5** Cross-sections of the DS 210 dry-air-operated sample-conditioning probe. (Courtesy of Columbia Scientific Industries).

## 15.3 WATER ANALYSERS

Water, the universal solvent, is everywhere around us. While additives can actually improve water quality for some uses, water impurities can cause corrosion and fouling of equipment and be a source of disease and pollution. Automated analysis is the key to solving critical water quality problems. Fast detection and correction of abnormalities are also important steps towards cutting treatment costs and keeping a plant in compliance with regulatory statutes.

Table 15.2 lists the different types of water most commonly used by humans and the processes typically applied to their purification. The parameters to

**TABLE 15.2** Types of water, features, processes to which they are subjected and parameters determined

| Type of water | Features | Process | Parameters |
|---|---|---|---|
| Untreated | Ground: high $O_2$ and sediment contents Deep: high $CO_2$ and solid contents; high hardness | Coagulation, clarification (chlorination) | pH, turbidity, chlorine |
| Drinking | Varying turbidity which determines treatment | Chlorination (fluorination) | Transparency, taste, odour, hardness, bacteriological activity (Fe, Mn) |
| Process | One-quarter of total industrial water | Chlorination | pH, hardness, corrosivity, litmus formation (Fe, Mn) |
| Feed | Higher boiler pressure. Higher purity required | Elimination of corrosivity and contaminants | Variable |
| Boiler | | pH/phosphate program, chelating agents, $O_2$ trappers | pH, Ca, Mg, $SiO_3^{2-}$, ($SO_3^{2-}$, $N_2H_4$, chelating agents, phosphate, etc.) |
| Purge | Drainage balance: security solid levels/heat and chemical losses | Drainage-recovery of chemical levels | $SiO_2$, $N_2H_4$, phosphates, pH, conductivity |
| Condensation | Dependent on the control results | Variable | hardness, conductivity, turbidity, $Na^+$, $SiO_3^{2-}$ |
| Cooling | Recirculated | Acidification, addition of phosphates, chromates and molybdates | Hardness, pH, additives |
| | Non-recirculated | Chlorination | |
| Waste | Two control lines: influent and effluent | Variable | pH, conductivity, phosphates, chlorine, dissolved $O_2$, alkalinity |

be controlled in these waters (shown in parentheses) are determined partly by the nature of the water and partly by the treatment to which it has been subjected.

There are other types of water and determinations not included in the table than require special solutions and are beyond the scope of this chapter. Nevertheless, some standard practices such as the determination of oxygen in its different possible forms and the speciation of elements are worth considering here. The large-scale surveillance of waterways as the starting point for the accomplishment of satisfactory levels of quality in drinking and industrial waters and the preservation of aquatic fauna is of utmost importance. Automated water surveillance stations are no doubt the best alternative for the realization of this task.

### 15.3.1 Off-line water analysers

The range of off-line instruments available for water analysis is wide. In fact, any analyser with optical or electrochemical detection can be adapted for this purpose. The use of liquid chromatography for the detection and quantitation of detergents or non-volatile organic compounds, of atomic absorption spectrometry for the analysis for heavy metal traces and of UV spectrophotometry for the determination of phosphates, nitrates and nitrites are representative examples of the potential utilization of conventional analysers for water analysis.

A common practice in water analysis is the indirect measurement of pollution from organic matter carried out by means of autoanalysers based on different principles.

The PW 9525 total oxygen demand (TOD) meter manufactured by Philips allows the measurement of TOD in about 2.5 min by burning all the oxidizable matter and expressing the amount of oxygen consumed in mg $O_2$/L. As can be seen from Fig. 15.6, the instrument consists of three essential parts: (a) the oxygen dosing and measuring system (two $ZrO_2$ cells); (b) the combustion system (an oven heated at 900°C); and (c) the injection system. A carrier gas ($N_2$) flows through the first $ZrO_2$ cell, which adds oxygen to the nitrogen stream at a constant rate, thereby providing a fixed $O_2$ concentration between 35 and 4000 ppm. The gas mixture is passed through an oven heated at 900°C and containing a special combustion tube furnished with a platinum catalyst. The gas is then passed through the second $ZrO_2$ cell, which has a measuring and dosing function. When a known volume of sample is injected into the oven, the water evaporates and the oxidizable matter is burnt by the oxygen in the $O_2$/$N_2$ mixture. The decrease in the $O_2$ concentration in the gas stream resulting from the combustion in the oven is detected and compensated for by the second cell. The

amount of oxygen required to restore the original $O_2$ level is the sought TOD
of the injected sample. The two $ZrO_2$ cells are no doubt the key parts of the
instrument. They are fitted with two internal porous Pt electrodes and two
external ring-shaped electrodes of the same material. The cells are heated at
about 600°C, at which $ZrO_2$ is a solid electrolyte. As an electrical current
passes from the inner to the outer electrodes, the oxygen is transported
through the wall of the zirconium tube. The amount of oxygen transported is
proportional to the magnitude of the current. The oxygen concentration can be
measured by using another property of electrolytic zirconium, *viz.* the
difference between the $O_2$ concentration inside and outside the zirconium tube
results in a voltage across the electrodes dependent on the partial $O_2$
pressure inside and outside the tube. In the first zirconium tube, the $O_2$ is
dosed in the carrier gas, while the second takes advantage of the two above-
mentioned properties of $ZrO_2$. The change in the $pO_2$ is measured as the voltage
drop across the measuring electrodes. Deviations from the preset $pO_2$ are
compensated for by a feedback system that doses or withdraws the carrier gas
in the second cell until the original concentration is restored. The current
required for the dosing is directly proportional to the amount of oxygen
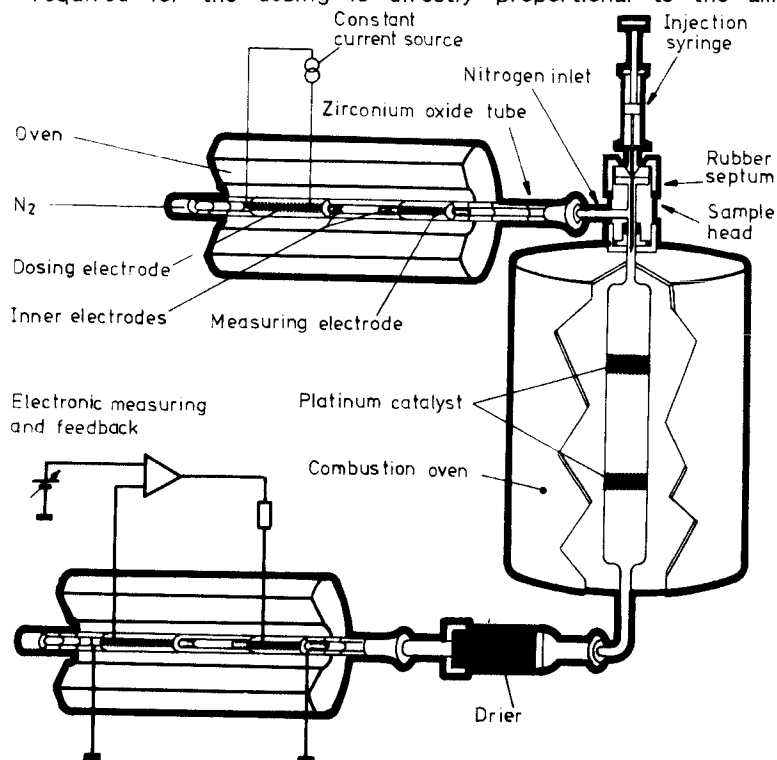


**Fig. 15.6** Scheme of the PW 9525 total oxygen demand (TOD) meter.
(Courtesy of Philips).

consumed in the combustion process. The instrument normally operates with manual injection of discrete samples, although it can be automated by means of a pump and an automatic injection device.

Another way of expressing the amount of chemically oxidizable matter is the so-called 'chemical oxygen demand' (COD), measurement of which can be carried out in an automatic fashion with the Aqua rator marketed by Precision Scientific. This instrument measures COD by causing organic materials to be oxidized on a platinum catalyst at 900°C by reduction of $CO_2$ to CO, yielding a mixture of both gases equalling the number of oxygen atoms required for the chemical oxydation:

$$C_aH_bN_cO_2 + mCO_2 \rightarrow (m+a)\ CO + (b/2)\ H_2O + (c/2)\ N_2$$
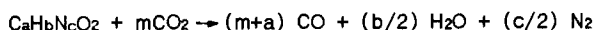
Hence, the CO readouts of the instrument are proportional to the COD and are safer when the number of oxygen atoms in the organic compound is equal to one-half of the number of hydrogen atoms. The samples (20 µL) are injected on to the catalyst and the oxidation products are carried along the instrument by means of a purified $CO_2$ stream.

The Metrohm 676 COD sample changer is based on the standard procedure for measurement of the amount of $K_2Cr_2O_7$ required to oxidize the oxidizable matter present in 1 L of water, so that the water sample is treated with excess Cr(VI) in a concentrated sulphuric acid medium containing $Ag_2SO_4$ as catalyst and heated at 175°C. The excess of Cr(VI) is determined by back-titration with Fe(II). The end-point is detected potentiometrically by means of a metal —ideally gold— electrode. The titrated solution is removed by suction and the titration vessel is washed with distilled water to make it ready for a new sample from the sampler, capable of holding up to sixteen pretreated samples.

Cnobloch *et al.* [36] have designed a relatively simple instrument for the analysis of groups of metal ions in natural and waste water based on electrolytic preconcentration and subsequent coulometric determination. The scheme of the instrument is shown in Fig. 15.7. The measuring cell contains two platinum electrodes and a stirrer which creates a thin, well-defined diffusion layer around the electrode, resulting in large response signals and hence in high sensitivity and reproducibility. The operational sequence is as follows. First, the measuring cell is filled with the waste water sample (pH 3.5); the metals are deposited on the cathode on application of a constant d.c. voltage and the cell is emptied. The cell is then filled with pure electrolyte (buffer solution of pH 3.5) and the metals are passed on to the solution by applying an inverse d.c. voltage. The electric charge consumed is a measure of the metal concentration in the waste water. The oxidation process is carried out

in pure water to avoid false response signals yielded by other oxidizable substances —particularly organic matter— potentially present in the sample. The polarity of the electrodes is reversed after each measurement, thereby ensuring their thorough washing. The number of metal ions that can be detected can be varied by selecting the appropriate deposition voltage, and so can the detection limit by changing the deposition time. The method developed by Cnobloch *et al.* is not intended for the accurate determination of heavy metals, but for that of groups. The apparatus acts chiefly as a control activating an alarm when a preset limit is exceeded. The plant operator is then in a position to take immediate counter-steps to avoid greater perturbations. The method does not replace other accurate methods for the determination of heavy metals in water; however, it limits the number of analyses to be carried out to those instances where critical values are reached or surpassed.
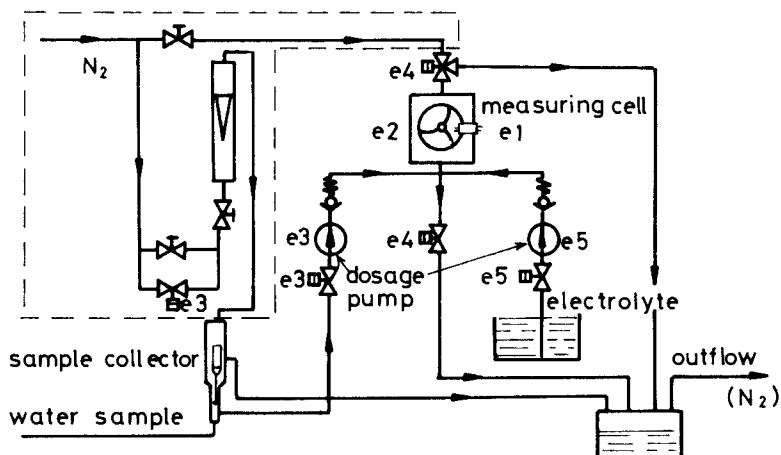


**Fig. 15.7** Schematic diagram of a heavy metal ion detector. (Reproduced from [36] with permission of Elsevier).

The automatic off-line speciation of elements in water has been implemented in a straightforward manner by flow-injection analysis with the aid of configurations adapted to the characteristics of the systems under study. Chromium has been speciated upon reaction between Cr(VI) and 1,5-diphenylcarbazide (DPC), which yields a coloured compound absorbing at 540 nm and free from interference from Cr(III). The manifolds used for this purpose allow the simultaneous or sequential determination of Cr(VI) and total chromium after oxidation of Cr(III) with Ce(IV) by use of a single- or dual-beam spectrophotometer. The configuration in Fig. 15.8a uses a single sample injection that is split into two channels reaching the sample and reference flow-cell, respec-

tively, of a dual-beam spectrophotometer (Fig. 15.8a1). The upper channel merges with a DPC current and yields a signal proportional to the Cr(VI) concentration in the sample upon passage through the flow-cell. The lower channel also merges with a Ce(IV) solution which oxidizes Cr(III) to Cr(VI) after merging with an indicator reagent; hence the signal obtained from the cell corresponds to the contribution of the two oxidation states initially present in the sample. A similar configuration with two flow-cells aligned with the light path of a single-beam spectrophotometer is shown in Fig. 15.8a2, while Fig. 15.8a3 depicts a configuration with merging prior to a single flow-cell. The sequential speciation of chromium can be carried out with the aid of a selecting valve ($V_2$) which allows or prevents the passage of a Ce(IV) stream, thereby facilitating the determination of Cr(VI) or total chromium by using two sample injections (Fig. 15.8b). All these configurations offer good mixture resolution with excellent reproducibility and determination ranges, which show the effectiveness of the methods used [37]. A configuration similar to that in
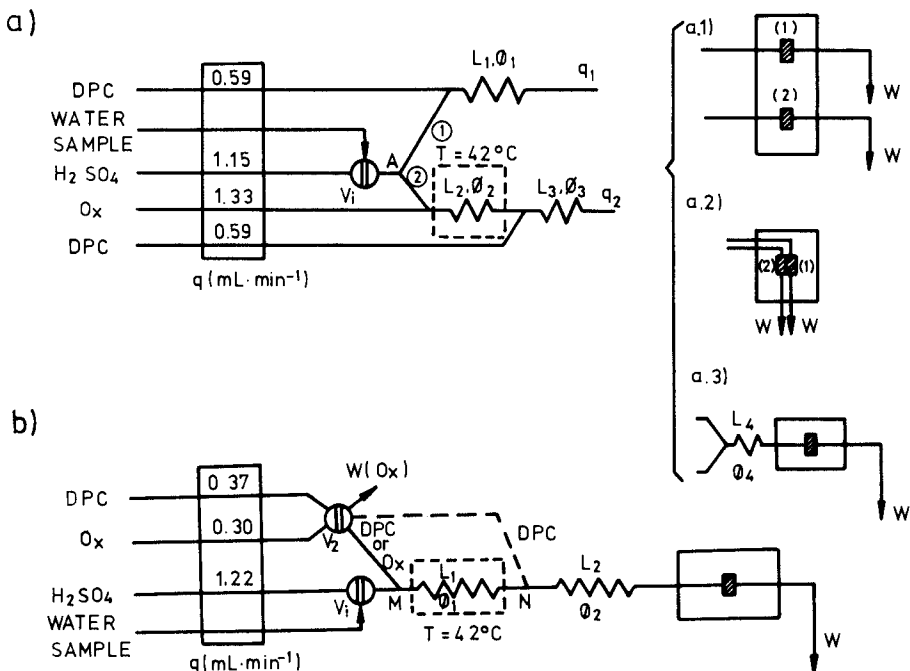


**Fig. 15.8** FIA configurations for the off-line speciation of chromium. (a) With a splitting point (A) and a dual-beam detector (a.1); with two cells aligned with the light path (a.2); with a merging point prior to a single flow-cell (a.3). (b) Sequential determination with the aid of a selecting valve. DPC: diphenylcarbazide; Ox: oxidant [Ce(IV)]; q: flow-rate; $V_1$: injection valve; L and $\phi$: reactor length and inner diameter; $V_2$: selecting valve; W: waste. (Reproduced from [37] with permission of Elsevier).

Fig. 15.8b has been used for the speciation or arsenic (as arsenite and arse-
nate) by use of the Molybdenum Blue reaction after complexation of As(V) with
Mo(VI). A $KIO_3$ solution is used to oxidize $AsO_2^-$ in this case. The calibration
garphs are linear in the range $10^{-6}-10^{-4}M$ for both ions and mixtures in ratios
up to 20:1 can be readily resolved [38].

### 15.3.2 On-line water analysers

This type of system features a number of advantages over off-line analy-
sers, namely sampling is more representative, the risk of contamination by the
containers or changes in the sample composition by storage is minimal and the
evolution of the system under study can be continuously monitored, which
results in more useful information.

#### *15.3.2.1 Single-parameter analysers*

There are a host of commercially instruments available for the monitoring
of a single parameter in both industrial and domestic water. All these instru-
ments are very similar and only differ significantly in the sensing system
used, which is suited to the analyte (parameter) to be determined.

*Colorimetric analysers.* The generic scheme of this type of instrument is
depicted in Fig. 15.9a. The water sample is introduced into the analyser by
means of the sample pump and mixed with a measured amount of reagent which is
introduced by its corresponding pump. The mixture then flows along the system
to the cell, where the colour development is monitored. The cell is the cylin-
der of the photometer pump and from it the sample-reagent mixture is pumped
through some delay loops around the mixing spool and out of the drain. As the
volumes delivered by the sample and reagent pumps are smaller than the capac-
ity of the photometer pump, some of the solution expelled by the photometer
piston's previous stroke is drawn back into the sample cell. A pinch valve as-
sembly functions to control the direction of the flow through the analyser.
This is accomplished by opening and closing tubes at the appropriate times in
relation to movements of the pump pistons. A flush system prevents the build-
up of reagent crystals in the upper part of the photometer block cylinder and
piston. Demineralized water, placed in the reagent compartment, is pumped
through the area of the sample cell that is above the photometer piston seal,
rinsing the area with each pump cycle (Fig. 15.9b). Also rinsed are the front
sections of the other pumps where the flush system water carries away any salt
that would increase the wear of the seal. Two flush pumps are mounted on the
pump panel to provide the energy to move the demineralized water through the
system. The inlet flush pump on the left side of the panel moves the rinse
water to the point where it enters the photometer block and the outlet pump of
the right draws it from the photometer block and pumps it out of the drain.
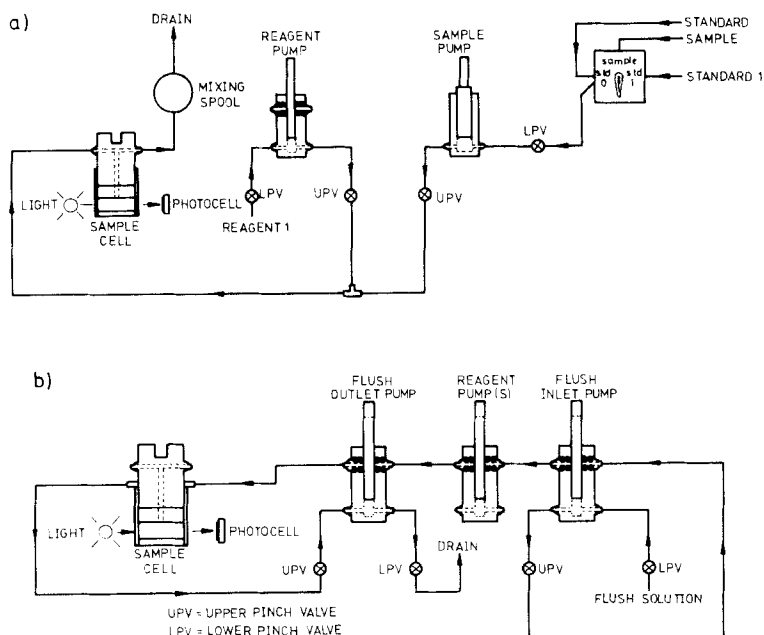
**Fig. 15.9** Sample and reagent flow diagrams (a) and flush (b) of a single-parameter water analyser with colorimetric detection. (Courtesy of Hach Co.).

Hach manufacture a series of single-parameter analysers with the above-mentioned features for the determination of copper (Model 61700), hexavalent chromium (Model 31700), permanganate (Model 31400), high-range silica (Model 61500), phosphate (Model 31500), ozone (Model 32000), free chlorine (Model 61100), chlorine dioxide (Model 31600), hydrazine (Model 32100), phenolphthalein alkalinity (Model 61400), total alkalinity (Model 61200), chelant (Model 61300), hardness (Models 31000 and 61000), etc. On the other hand, the trace analysers marketed by Hach are gravity-based and control the sample-to-reagent ratio by means of precisely selected capillaries located in the sample and reagent flow paths (Fig. 15.10). The temperature of sample and reagents is controlled during the mixing and reaction phase in order to ensure reproducibility. The temperature control is maintained because the head regulator and delay blocks are secured to a mounting plate that is an integral part of the sample heater. It is important, however, that the sample flow be relatively constant because wide fluctuations will affect temperature control. The water sample entering the analyser flows first through the sample heater, where it

is heated at 50°C, and then enters the head regulator, which provides a constant head above the water capillary at 5 mL/min. As it leaves, it mixes with reagent 1 and drops into the first delay block, where a delay allows the first step of the reaction to take place. This mixture then drops into the second delay block, where it is mixed with reagent 2, which produces the monitored product. This final mixture is detained in the second delay block for several minutes to ensure that the colour can develop fully before it flows on the sample cell for measurement. This is the operational basis for the trace silica (Models 651C and 1234D) and trace phosphate (Model 2359C) analysers.
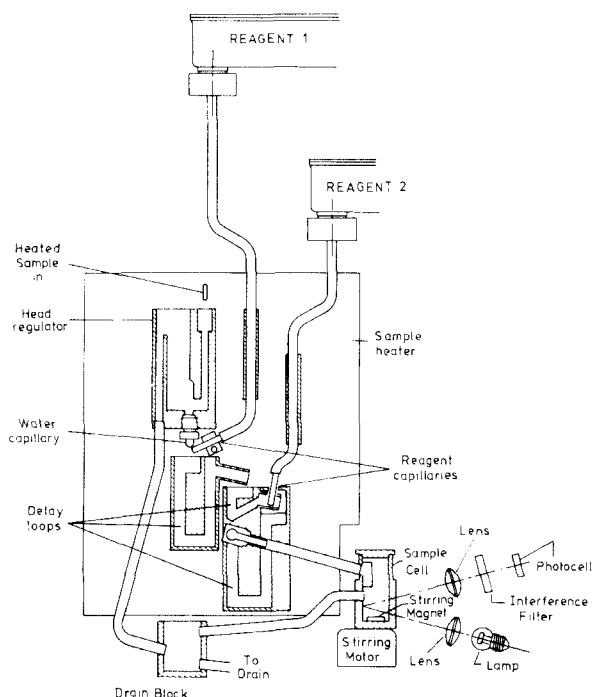


**Fig. 15.10** Scheme of colorimetric gravity-flow autoanalyser. (Courtesy of Hach Co.).

A colorimetric analyser for the determination of phenol in waste water involving prior distillation of the sample by means of a home-made device has been reported by Goodwing and Marton [39]. Figure 15.11a shows the distillation unit and Fig. 15.11b its connection to the analyser. The distillation assembly consists of a distillation coil, a condenser and a heat exchanger.

The distillation coil has an expansion chamber partially filled with 1-mm glass beads, a bottoms draw to remove hot acid and an overhead draw to remove vapours. The cycle time includes 1 min for sampling and 3 min for washing.
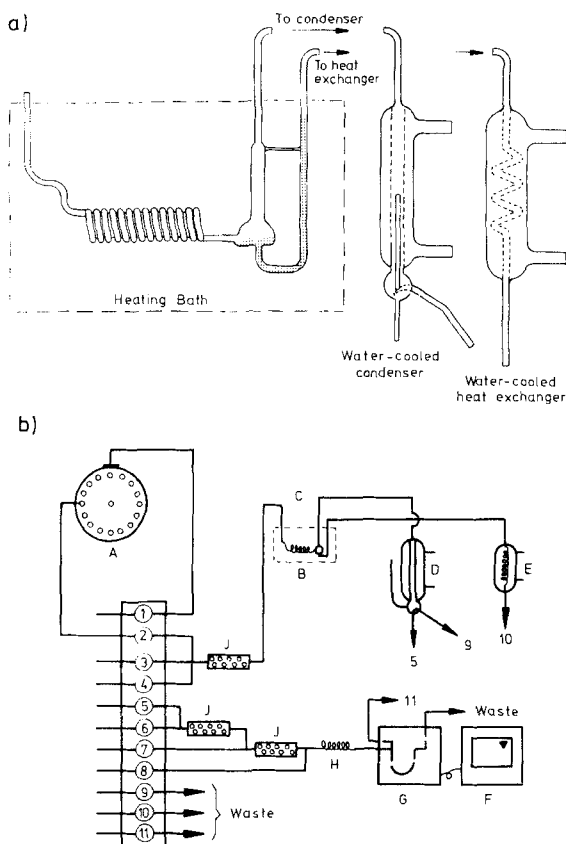


**Fig. 15.11** Colorimetric phenol analyser. (a) Details of the distillation unit, condenser and heat exchanger. (b) General scheme of the instrument. A, sampler; B, heating bath with heating rod; C, distillation head; D, condenser; E, heat exchanger; F, strip-chart recorder; G, colorimeter; H, coil; J, single bead-string reactor; K, peristaltic pump; (1) wash water; (2) sample; (3), air; (4) phosphoric acid; (5) overhead condensate; (6) 4-AAP reagent; (7) Fe(III) reagent; (8) air; (9) level control; (10) bottoms draw; (11) debubbler draw. (Reproduced from [39] with permission of Elsevier).

*Turbidimetric.* There are two basic types of on-line water turbidimeters: surface scatter and flow-through. In turbidimeters of the former type the sample flows into a constant-level well. The light beam is directed to the sur-

face of the cup and the radiation scattered by the particles in the liquid surface is measured at a specific incidence angle. This type of instrument has the advantage that the optical elements are never in contact with the sample. In the latter type of instrument, the sample flows through a tube and the light impinges on it through an optical window located in a lake or at the end of the tube, after which the scattered light is measured. The Model 5 surface scatter turbidimeter and the Model 1720B flow-range process turbidimeter marketed by Hach are representative examples of each type of turbidimetric on-line water analyser.

*Dissolved oxygen detectors.* These consist essentially of an electrochemical cell with a gold cathode and a silver anode immersed in an electrolyte solution separated from that of the sample by a chemically resistant polymer membrane permeable to the gas, so that part of the dissolved oxygen in the sample proportional to its partial pressure diffuses across the membrane and is reduced in the cathode, giving rise to a current proportional to the oxygen concentration in the sample. The PW 9600 dissolved oxygen transmitter and PW 9610 dissolved oxygen sensor manufactured by Philips include a transmitter unit which accommodates two printed circuit boards —one of which provides the measuring circuitry— with solid-state components. Both instruments have alternative housings for the sensing element. The immersion probe or flow assembly used depends on the particular requirements of each installation. The two instruments provide continuous automatic measurements of dissolved oxygen in water, fully compensated for the sample temperature.

*Organic carbon analysers.* A variety of procedures have been reported for the automated determination of carbon-containing compounds in water by oxidation with peroxydisulphate and the aid of UV radiation or Ag as catalyst. In 1969, Erhardt [40] proposed an automated procedure for the analysis of sea water by oxidation with peroxydisulphate under UV light. The $CO_2$ produced was absorbed by an alkaline solution and the resulting change in conductivity was a measure of the concentration of dissolved $CO_2$. Another method, developed by Goulden and Brooksbank [41], used UV irradiation or an Ag catalyst to effect the oxidation of the samples and measured the $CO_2$ generated by means of an IR analyser. Princz *et al.* [42] also developed a less common method for the determination of COD based on potentiometric measurements. The instrument, depicted in Fig. 15.12, has an autosampler with alternate samples and blanks, a UV tube reactor, a peristaltic pump and a flow-cell with a $CO_2$ gas electrode. The practical procedure involves the prior separation of inorganic carbon by addition of $H_2SO_4$ to the sample up to pH 2-3, followed by stripping of the evolved $CO_2$ with $CO_2$-free air. The pretreated solution is reacted with the oxidant ($K_2S_2O_8$) and introduced into the sampler, from which it is passed through the

reactor tube, where organic carbon is oxidized and measured in the controlled-temperature flow-cell. The generated emf is recorded analogically by the log-graph device.



$CO_2$
ELECTRODE

AUTO
SAMPLER

UV
REACTOR

SAMPLE
&
BLANK

PUMP

mV METER
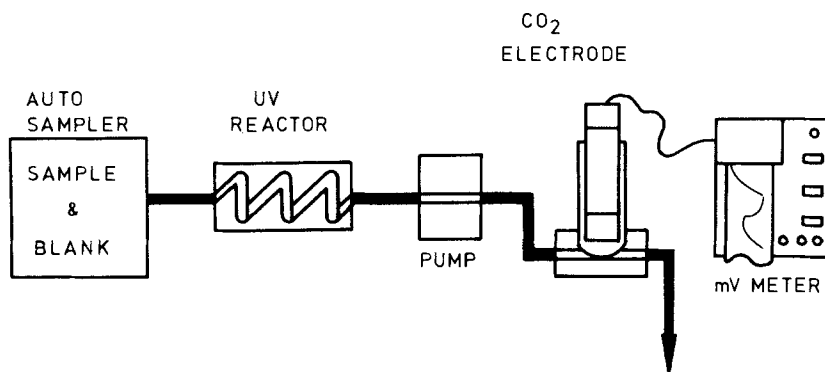
**Fig. 15.12** Potentiometric COD analyser. (Reproduced from [42] with permission of Elsevier).

The Technicon Monitor IV is a segmented-flow analyser for the continuous on-line monitoring of COD, TOD and TC. For the determination of TOD and COD, the sample is acidifed and sparged to remove organic carbon, and later mixed with $H_2SO_4$ and $K_2S_2O_8$ and subjected to UV light in a UV digester. The $CO_2$ generated is isolated from the matrix by means of a dialyser with a gas-permeable silicone rubber membrane. The acceptor stream contains phenolphthalein dissolved in a carbonate-hydrogen carbonate buffer, the change of colour resulting from the absorption of $CO_2$ being monitored by a colorimeter. The reaction sequence for the determination of each parameter is illustrated in Fig. 15.13a. The sparging step is omitted in the determination of total carbon, which is measured in a direct manner. The sparging of the samples for removal of inorganic material may cause some organic compounds to be lost, particularly when volatile or water-insoluble substances are present. These losses can be evaluated by comparing the readings obtained with and without sparging in an acidic medium —the acidified samples will provide a lower concentration for the inorganic carbon than that actually present. As a rule, waste water rarely contains volatile or very insoluble materials, which are more likely to be found in other types of water. The essential components of the instrument, shown in Fig. 15.13b, are : (1) overflow sampler; (2) solenoid valve, which facilitates the introduction of the sample or the reference solution into the manifold; (3) proportioning pump; (4) UV digester; (5) manifold assembly, with

a)
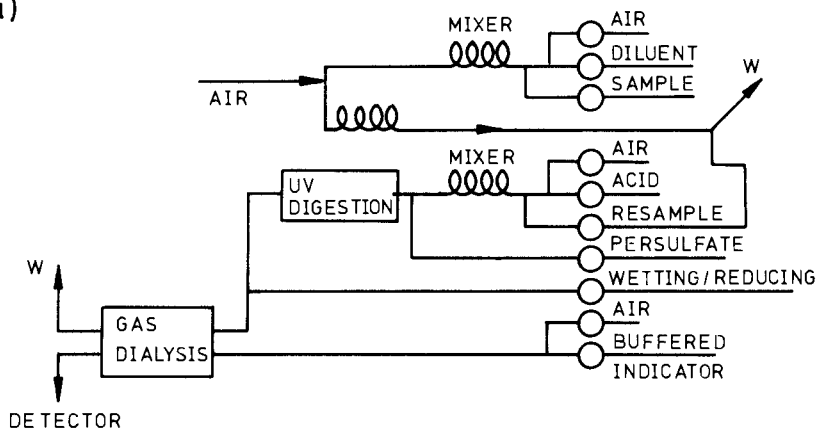


b)
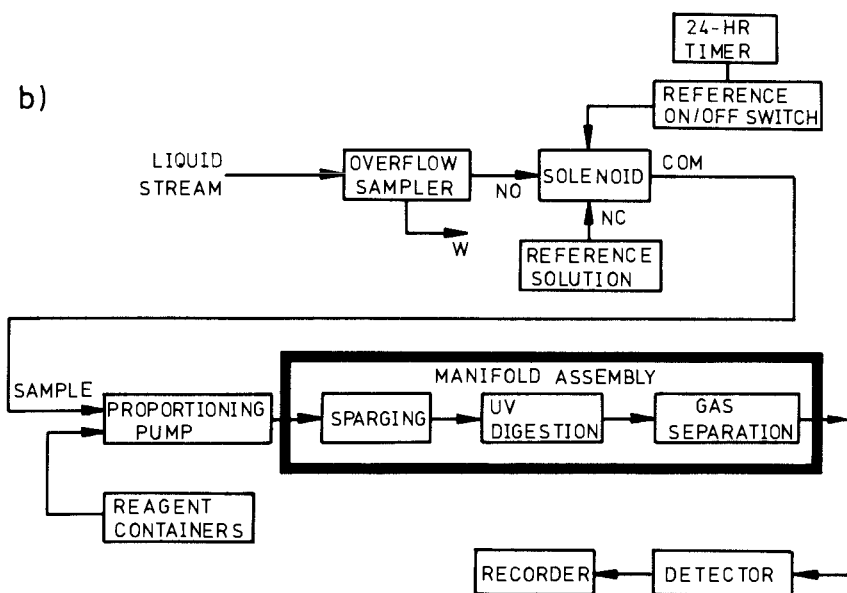


**Fig. 15.13** Monitor IV system. (a) Reaction sequence. (b) Block diagram of the interchangeable module design. (Courtesy of Technicon).

the required glassware, tubing, heating baths, dialysers and fittings —this unit is intended to facilitate manifold changeover for the analysis of different analytes—; (6) detectors —the analyser can use an ISE or colorimeter depending on the parameter to be assayed—; (7) recorder (the Monitor IV provides direct analogue data output and strip-chart printouts for permanent record of the analytical values; and (8) sparging system, consisting of an impingement pump, sparging coil and an additional gas-liquid separator. The sparging air is supplied by an air pump and purified from organic contaminants by passage through an active carbon filter. The sparging coil is by-passed for total carbon measurements. The instrument optionally allows:

(a) Dual sample analysis. A timer and a programmable valve permit the alternate analysis of two streams with auto-correction (standardization) at preset intervals. An auto-correction feature is provided for monitoring of applications involving drifts arising from system changes such as reagent degradation or pump tube delivery changes, and electronic long-term drifts. Depending on the expected nature of the drift, the auto-correction unit may be set to correct for either the baseline drift or sensitivity drift.

(b) Use of high-low alarms. This module warns the operator about any abnormal change in the concentration of the parameter being determined.

(c) Use of a continuous water clarifier. A module which provides samples from which particles with diameters greater than 0.5 μm have been filtered out for sample streams.

### 15.3.2.2 Multi-parameter analysers

The legislation on water pollution and quality control necessitates analyses for an increasingly larger number of parameters. These demands have been met by commercial firms with the manufacture of multi-parameter analysers. The Philips Environmental Protection range is representative of this type of instrument.

The PW 9835 Automatic Water Monitor allows the measurement of up to six water quality parameters (pH, pCl, redox potential, conductivity, dissolved oxygen and temperature) in ground waters in a continuous automatic fashion. The period of unattended, maintenance-free operation may be as long as 1 month or more, depending on local conditions. Two important features contributing to accurate, unattended operation are automatic sensor cleaning and automatic calibration of the instrument. The sensors can be kept from fouling by algae and other materials normally present in ground water by an optionally available automatic ultrasonic cleaning unit, which operates periodically at preset time intervals. Automatic calibration provides proof that the automatic water monitor is working properly. Reliable reference values are given daily and

truly correct measuring data over the full unattended period are assured. A turbidimeter can be optionally included. The autoanalyser consists of two distinct parts, an upper electronic section and a lower wet section. The electronic section consists of three potentiometric systems for measurement of pH, pCl and redox potential, a conductimetric detector, a voltammetric system for measurement of dissolved oxygen, a temperature transmitter and a control unit effecting the following functions:

(a) Control of the sequence for the calibration process. This includes timing the motion of the slides in the measuring block, filling the individual compartments in the measuring block with fluid and flushing. The calibration cycle takes 41 min.

(b) Generation of electrical and visible status signals (0 or 15 V) and acceptance of incoming signals for remote control.

(c) Control of the sampling pump and hence of the sample stream through the monitor.

(e) Supply of +24 and −24 V d.c. power for the measuring transmitters.

(f) Thumbwheel adjustment to allow the user to select the time at which a calibration cycle should start automatically. This is adjustable from 0 to 16 h subsequent to setting.

(g) Fault detection in case the water pressure inside the measuring block becomes too high, in which event the sample pump is turned off; the air pressure for pneumatic operation is too low, in which event the monitor is switched off; power consumption by the sampling pump is too high, in which event it is switched off; or the sliding valves in the measuring block are positioned improperly.

The electronic unit is completed by recording equipment which can be from a multi-channel recorder to up to four dual-line recorders. Data transmission is achieved by mounting the appropriate equipment in the recorder section. It is also possible to mount a microcomputer-based pollution data reductor.

The wet section includes: (1) a PW 9825 flow-through measuring block into which the appropriate sensors are mounted; (2) bottles containing electrolyte for replenishing the contents of double-junction reference electrodes; (3) pneumatic devices consisting of pressure-reducing valve tubing and pneumatic valves; and (4) a number of 5-L containers for calibration liquids, placed in the bottom of the cabinet. In the measuring step, the PW 9825 block functions as an ordinary pipe through which the sample flows at a rate of 1–2 m/s. If an ultrasonic transducer is needed, it is placed before the electrodes, which are also mounted in the block. During calibration, the block is flushed and drained, and the water supply is switched off. The sliding valves mounted in the block are pushed upwards so that the pipe is divided into measuring cham-

bers. The calibration liquids are then dosed pneumatically into these chambers. Overflow pipes allow for the elimination of air and excess calibration liquid. After 20 min, the sliding valves are pushed downwards, the pump is switched on and the block is flushed. A second calibration is performed in the same manner, with the second calibration liquid dosed to the chambers by means of their respective pneumatically operated valves. After the second calibration, the water monitor is switched to the measuring cycle. Calibration of dissolved oxygen is carried out by exposing the sensor to air. Dirt and superfluous water on the membrane are removed by an air stream during the first few seconds of both calibration cycles. All positions and modes of the measuring block are indicated by small semiconductor lamps on the control panel.

Comber and Nicholson [43] reported a home-made design for the continuous monitoring of rivers and estuaries. The instrument allows the detection and electrochemical quantitation of cyanide, sulphide, ammonia and pH (ISEs), as well as salinity, dissolved oxygen and temperature (Fig. 15.14). The sample
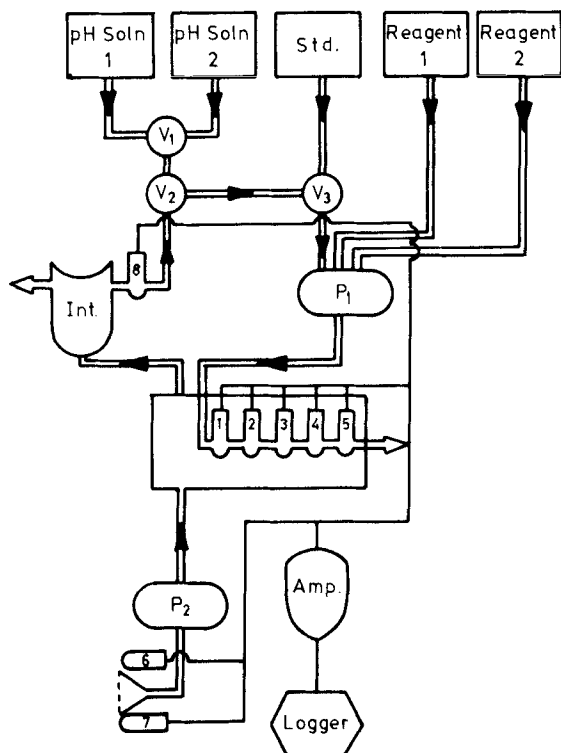


**Fig. 15.14** Flow diagram of continuous water monitor. Electrodes: 1, cyanide; 2, sulphide; 3, ammonia; 4, pH; 5, reference; 6, salinity; 7, oxygen; 8, temperature. V: valve; P: pump. (Reproduced from [43] with permission of the Royal Society of Chemistry).

intake can either be mounted on a metal pole fixed to the bow of a vessel —approximately 1 m below the surface— for use in horizontal profiling of a river course, or it can be lowered through the water column for depth profiling. The dissolved oxygen and salinity probes are mounted alongside the sample intake and are calibrated in the laboratory. The water is pumped on the boat at a rate of approximately 500 gal/h by using an on-board pump ($P_2$). The power for this pump and the remainder of the equipment is derived from the vessel's accumulator, either directly or via an electronic inverter to provide 240 V and 50 Hz. Later developments extended the scope of this instrument to analyses for metals by anodic stripping voltammetry and ion exchange, the determination of nitrites and nitrates by FIA and on-line research on organic compounds with the aid of a fast-scan monochromator.

The principle behind the FIA technique (reversed mode) has been exploited to develop a completely automated instrument [44] (Fig. 15.15) in which the sample is continuously pumped along the system and in which different injection valves [45] or a single injection valve aided by a selecting valve [46] are used to introduce the reagents required for the determination of each analyte in the appropriate sequence and at the required time intervals. The reacting plug is driven to the flow-cell of a photometric detector, and the
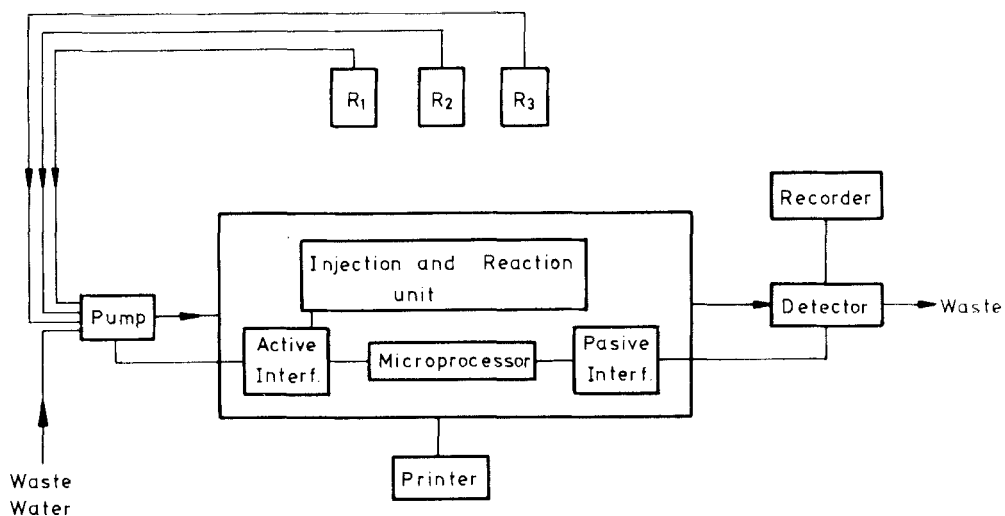


**Fig. 15.15** Completely automated instrument for the determination of pollutants, based on the reversed FIA principle.

signal yielded is recorded or acquired by a microcomputer through a passive interface and later processed. The active interface also used allows actuation of the pump and the injection valve(s), so that, by use of a suitable program, the instrument is started at the required time intervals, injects reagents according to the preset sequence, collects data provided by the detector and compares them with the calibration graph for each analyte stored in the program. A tolerated limit is also stored for each analyte, so that if the concentration of any of them surpasses the limit, an alarm system warns the operator. This analyser allows the quantitation of anions ($CN^-$, $NO_2^-$, $S^{2-}$) and cations (Al, Fe, Cu) [46]. The incorporation of a glass–calomel electrode prior to the injection system along the sample channel allows the determination of pH [47].

The reversed FIA (rFIA) mode also allows for the on-line speciation of elements. Thus, different rFIA configurations (Fig. 15.16) have been used for the speciation of chromium based on the indicator reaction between Cr(VI) and 1,5-diphenylcarbazide (DPC). Figure 15.16a shows the configuration used for the completely continuous monitoring of Cr(VI) and periodic measurement of Cr(III). The sample circulates continuously along the system and merges with an acidic DPC stream, its evolution in the water being continuously monitored. At time intervals dependent on the required frequency, Ce(IV) is injected to oxidize Cr(III) to Cr(VI), which yields a signal proportional to the Cr(III) concentration initially present in the sample [48]. The configuration in Fig. 15.16b consists of two sub-systems for the determination of Cr(VI) (1) and total chromium (2). The selecting valve, SV, allows the arrival of one or another stream to the detector and hence the obtainment of the Cr(VI) or total chromium concentration, the Cr(III) concentration being calculated by difference [48]. The configuration in Fig. 15.16c uses both rFIA and the asynchronous merging zones mode for the speciation. The dual injection valve used simultaneously inserts into the system a large volume of DPC and a small volume of Cr(VI), so that the latter merges at point A with the tailing portion of the DPC plug, yielding a first signal corresponding to the reaction of Cr(VI) alone in the heading portion of the DPC plug and a second, larger signal corresponding to the tailing portion of the plug, where Cr(III) has been oxidized and therefore contributes to the analytical signal [49].

An rFIA–asynchronous merging zones configuration has be used for the speciation of up to nine different chromium forms —aquo complex, mono-, di- and tetrahydroxylated Cr(III) and molecular, anionic and dimeric Cr(VI). It includes a glass–calomel microelectrode inserted in the sample stream prior to the merging with the reagents, and a microcomputer which acquires the measured pH and chromium concentrations —Cr(VI) and total Cr. These data are processsed by a computation program in which the equilibrium constants of the

a)



b)



c)
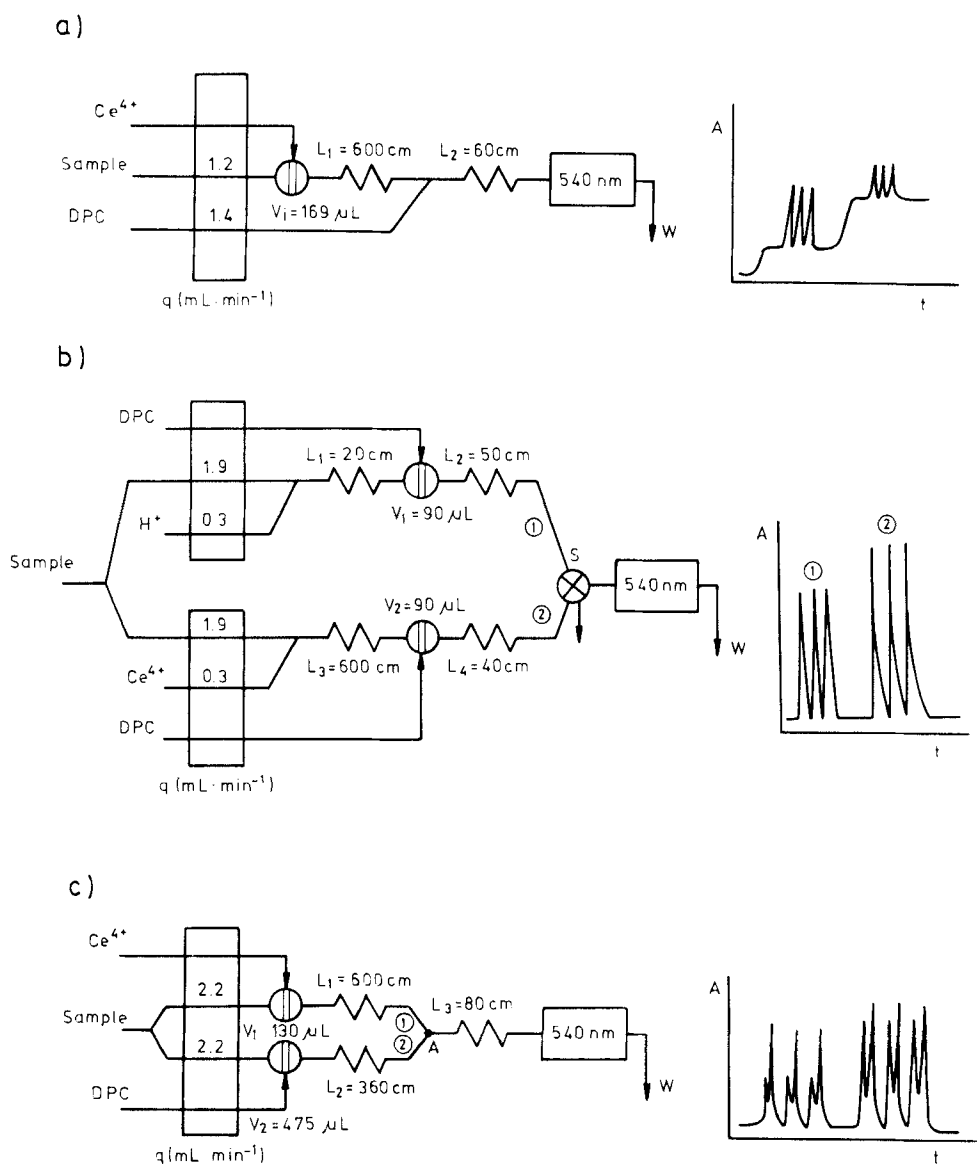


**Fig. 15.16** rFIA configurations for the speciation of Cr(VI) and Cr(III). (a) Completely continuous determination of Cr(VI) and periodic measurement of Cr(III). (b) Sequential method for this speciation by use of a selecting valve for determination of Cr(VI) (channel 1) and total chromium (channel 2). (c) Asynchronous merging zones for simultaneous determination of both species. q: flow-rate; $V_i$, $V_1$ and $V_2$, injection valves; S: selecting valve; W: waste. The recordings obtained are shown to the right. (Reproduced from [48] with permission of Springer Verlag).

nine above-mentioned species are stored and which yields the concentration of each [50].


## 15.4 AIR ANALYSERS

Atmospheric pollution, on account of its special features, is the most important aspect of environmental pollution. The most recent trends in this field point to atomic absorption and emission techniques (metals) [51], the use of GC and HPLC for organic compounds [52-55] and the development of new electrochemical sensors [56-60] facilitating off and on-line measurements. *In situ* measurements, carried out by remote sensors [60,61], allow the detection and quantitation of air volumes in the atmosphere at a distance from the instrument without the need for sample collection. Measurements are mostly based on the optical properties of matter (light absorption, emission or scattering) and the instruments used can be located in a fixed position or on a moving platform, either on the ground or in the atmosphere (planes, balloons, satellites). Two general types of light sources can be distinguished depending on whether they use passive or active sensors. Passive sensors use natural light and depend on the intensity, spectral distribution, etc. of the source —light coming from the sky or from the sun itself after passing through the atmosphere. They are often used in the limb sounding configuration, looking at sunrise or sunset through the whole atmosphere to increase the integrated signal over very long paths. In that case, they measure a quantity which is the product of a concentration and a distance. Passive sensors can be classified into radiometers, ultraviolet vidicons, spectrometers, interferometers, Fourier transform analysers and laser heterodyne amplifiers of spectral lines. Active sensors have built-in light sources —generally lasers— and are obviously more complicated and expensive than passive sensors. The source can be at a long distance from the detector (hundreds or thousands of metres), and the light collected by the telescope allows the measurement of the total amount of a gas along the light path rather than its concentration. Most passive detectors can be used as active detectors by fitting them with a light source. The commonest are laser absorption spectrometers and LIDARs (monowavelength, differential, Raman or fluorescence). Active and passive sensors are intended for different applications. Passive instruments are better suited to stratospheric observation in balloons or satellites because of their compactness and low energy consumption. At ground they can provide a huge amount of information on the nature of chemical compounds. Active sensors have a much higher spatial resolution and should be more useful for studying the atmospheric phenomena from the ground to a distance of about 5-10 km. The chief

drawbacks of these instruments arise from the difficulties in interpreting the complete information provided by the data; not only are the spectral data often confusing, especially in the lower frequency region (infrared, micro-wave), but also the atmospheric parameters are changing so much and so quickly in space and in time that very great care is necessary not to adopt simplistic assumptions causing large errors [62].

The problems associated with the sampling and automation of this stage were dealt with in their corresponding sections, so this section is exclusive-ly devoted to the automated instrumentation available for this type of sensor. As with water analysers, air analysers are classified into off- and on-line analysers, and the latter in turn into single-parameter and multiparameter.

### 15.4.1 Off-line air analysers

Off-line systems are typically used for the detection of the metal ana-lytes occurring in the atmosphere as particulates or fumes which, after collec-tion by an appropriate filter, are subjected to dry ashing followed by acid decomposition [63] or, more commonly, wet ashing [63], and quantitation by ICP-AES [64], flame AAS [65], normal or furnace AAS [66,67], or X-ray fluor-escence spectroscopy [68]. Oguma and van Loon [69] reported a method for the

a)



b)
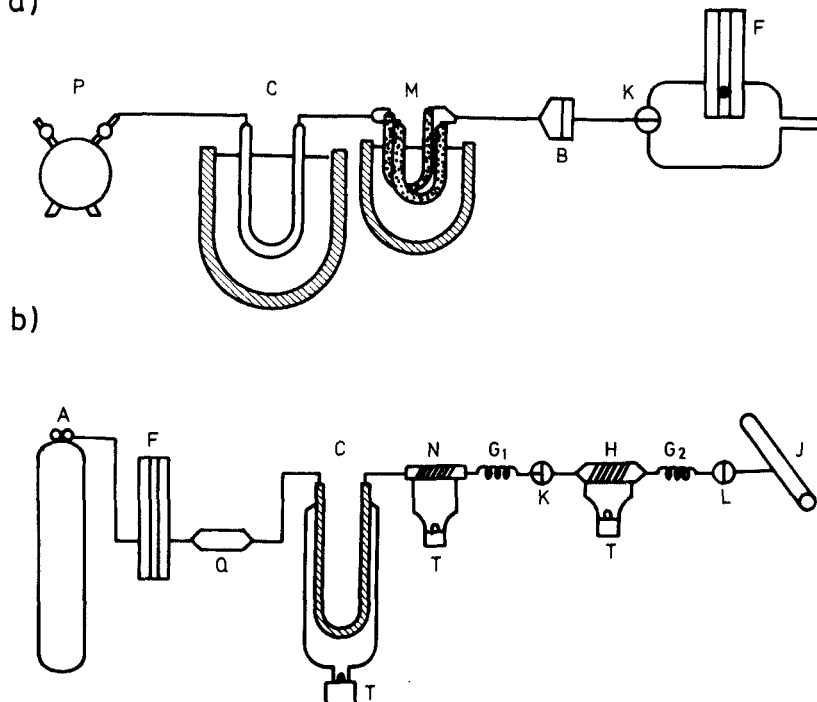


**Fig. 15.17** Assembly for determination of vapour mercury in air. (a) Sampling apparatus; (b) measuring instruments.

determination of total mercury vapour in air by AAS using the apparatus depicted in Fig. 15.17a for sample collection. Air is pumped through a 0.45-μm Millipore filter (B), the moisture traps (M) and the collection tube (C), in this order, at a rate of 2 L/min, checked by the passage of the air through a flow-meter (F) by turning the three-way stopcock (K) at 10-min intervals. After an appropriate sampling time, the collection tube is dislodged from the system and installed in the measuring system (Fig. 15.17b), where it is warmed to room temperature and then heated at 100-115°C for 5 min. During these two stages, a nitrogen stream is passed through the collection tube, the pyrolyser (N), the cooling coil (G) and the amalgamation tube (H), in this order, at a flow-rate of 0.5 mL/min. Then stopcocks K and L are shut off and the amalgamation tube is heated at 500°C for 30 s. The mercury vapour thus released is swept into an open-ended glass tube (20 cm x 1.5 cm I.D.) with a 5 mm I.D. inlet tube fused in the middle that is placed in the burner head of a Perkin Elmer 305B atomic absorption spectrometer and aligned in the usual manner to allow maximum light from the hollow-cathode lamp to reach the detector. The height of the recorded peaks is used to determine mercury. The analytical system is standardized at a known temperature by means of a septum inserted between the cooling coil ($G_1$) and the stopcock (K).

### 15.4.2 On-line air analysers
#### *15.4.2.1 Single-parameter analysers*

The on-line single-parameter instrumentation for the determination of gases employs a variety of detection systems.

Good proof of concern about the pollution caused by sulphur dioxide is the large number of analysers developed for the continuous monitoring of this parameter. The literature review by Hollowell *et al.* [70] on the instrumentation for continuous monitoring of $SO_2$ deals with the different detection techniques used for this purpose, namely conductimetry, colorimetry, coulometry, electrochemical transducing, flame photometry alone or in conjuction with gas chromatography, non-dispersive and dispersive absorption and condensation nuclei formation. Recent studies were oriented to the resolution of the problem of real-time on-line analysis for $SO_2$ at low concentrations [71] by means of a dispersive infrared detector or a coated piezoelectric mass transducer [72]. The device used in the latter case, Fig. 15.18, allows the rapid variation of the crystal environment in terms of the gas composition, temperature and water composition of the gas stream. The oscillator and frequency meter board of the interference rack are controlled by a microcomputer.

Most commercial instruments available for the determination of $SO_2$ are based on the UV light excitation of the $SO_2$ molecules in gas mixtures [73] and

the measurement of the resultant fluorescence (e.g. Model SA700 Fluorescence SO₂ analyser from Columbia Scientific Industries Corporation) or of the light emitted by the sulphur species in passing through a hydrogen-rich flame (Sulphur Dioxide analyser from the same company).
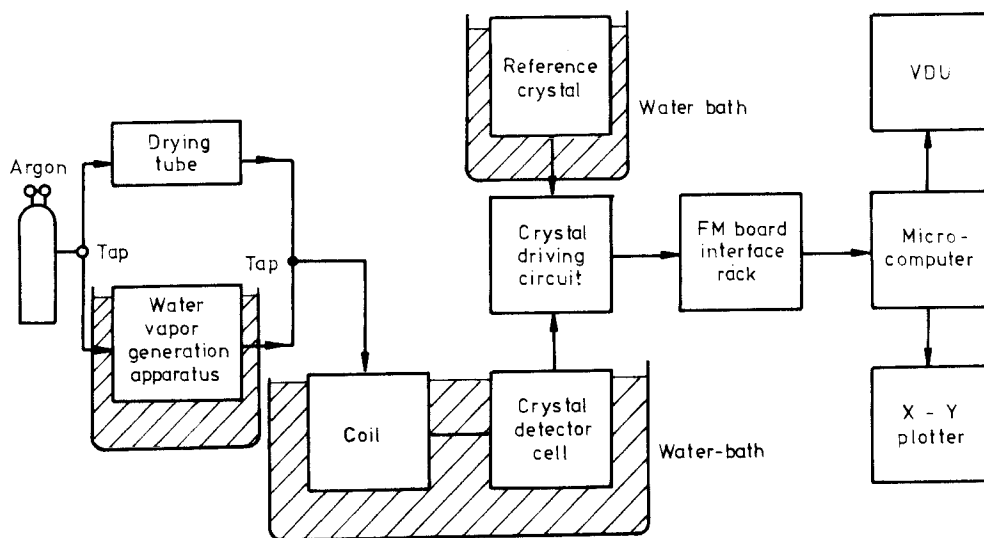


**Fig. 15.18** Scheme of experimental instrument for on-line measurement of SO₂ with detection by a piezoelectric crystal sensor. (Reproduced from [72] with permission of the Royal Society of Chemistry).

On the other hand, the single-parameter gas analysers manufactured by Philips (PW 9755 SO₂ monitor, PW 9780 H₂S monitor, PW 9775 CO monitor, PW 9760 NO monitor and PW 9765 NO₂ monitor) are based on continuous coulometric titrations (Fig. 15.19). The air is aspirated from the atmosphere via an air sampler and dust filter and is then pumped at a constant flow-rate through the monitor where the analyte concentration is measured. On entering the monitor, the air is first passed through a chemical scrubber to filter out unwanted components that might affect the analyte reading. The filtered air is then passed at a constant rate through an aqueous solution of KBr, Br₂ and H₂SO₄ in the thermostated cell. By use of the two electrodes immersed in the electrolyte, the free bromine concentration is converted to a redox potential which is compared with a known reference potential. A control amplifier senses

the difference between these two voltages and sets up a flow of electric current in the electrolyte via two more electrodes. This electric current converts bromide ions to free bromine, whose concentration in the electrolyte is therefore restored. As the extent of reduction depends on the amount of analyte ($SO_2$ or $H_2S$) passing through the electrolyte, the electric current used will be directly proportional to the amount of analyte present in the sample. By keeping the air flow-rate constant with the aid of a vacuum pump and a thermostatically controlled critical orifice, the analyte concentration in the air can be readily calculated. After leaving the electrolyte, the air is passed through a convection-cooled Peltier cooler. The water evaporated from the electrolyte solution is carried by the air stream and is condensed in the cooler, falling on the electrolyte solution, the level of which is therefore kept constant. The measurement, zero-checking and span-checking functions can be automatically selected by means of a three-way motor-driven valve operated under remote control. In the measurement position, air from the sampling system (and dust filter) passes only through the chemical scrubber on its way to the measuring cell. When the valve is set to the zero-checking position, the air is sucked through an active charcoal filter to remove the analyte before reaching the chemical scrubber. The purified air then enters
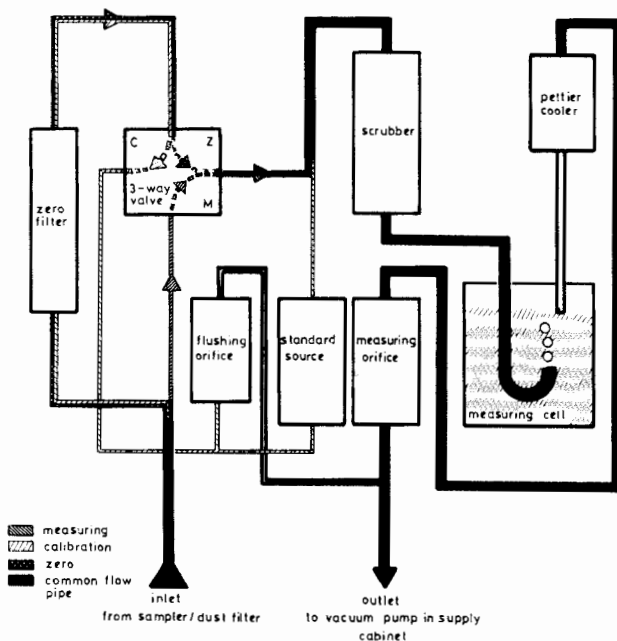


**Fig. 15.19** Block diagram of gas analyser with coulometric detection. (Courtesy of Philips).

the measuring cell and gives rise to an output signal which defines the zero level. With the valve in the span position, the above-described procedure is the same as that between the zero-filter and the chemical scrubber, the air passing through a standard source where it is dosed with a known amount of analyte. The standard source consists of a permeation tube with liquid $SO_2$ and $H_2S$ thermostatically maintained at a constant temperature.

In the CO monitor, the air, after passing the scrubber, enters a column where it reacts with $I_2O_5$ at 160°C to yield free iodine. This reaction is necessary on account of the electrochemical inertia of CO. In this manner, as the reaction efficiency is constant, the amount of $I_2$ formed will be directly proportional to the CO concentration in the air. The $I_2$ vapour is passed through a measuring cell with two electrodes which contains the electrolyte and is kept at 37°C. On entering the cell, the vapour is passed through a graphite cathode, where iodine is reduced to iodide and an electric current proportional to the amount of iodine and hence to that of CO in the air is generated. The current flow causes a potential difference between the two electrodes which is measured by a potential amplifier. All other stages of the process are the same as those taking place in the $SO_2$ and $H_2S$ analysers. The Philips NO and $NO_2$ monitors  also correspond to the scheme in Fig. 15.19. After isolation from potential interferents, NO must be oxidized to $NO_2$. The air stream is passed through a KI solution which reacts with the $NO_2$ to yield iodine, which gives the measured current upon reduction.

A home-made analyser with a self-scanned photodiode array as a multiplex sensor has been used for laboratory detection and measurement by dispersive spectroscopy of trace amounts of polluting $NO_2$. The on-line data acquisition and numerical analysis system allows the elimination of some systematic errors and drifts (Taylor filtering) and the noise associated with high spatial frequencies (low-pass filtering). The experimental set-up used is shown in Fig. 15.20. The white light source, S, illuminates slit $F_1$ homogeneously. Two spherical mirrors $M_1$ and $M_2$ with a focal distance of 15 cm for the image of $F_1$ on the slit ($F_2$) giving access to the dispersion system (an array of 1200 lines/mm). Tube T, filled with $NO_2$ at different partial pressures, intercepts the beam betwen $M_1$ and $M_2$. Lens $L_1$, diaphragmed by D, forms the image of $F_2$ onto the microphotodiodes (μPh). A cylindrical lens, $L_2$, allows the light to be concentrated by a factor of 10 in the direction perpendicular to the line. The 1728 photodiodes (15 μm spatial period and 16 μm length) are cooled by a Dewar vessel  containing a mixture of methanol and dry ice. The electric signals from the detection system are acquired by a computer. The gathered data allow the calculation in real time and for each diode the mean and variance of n steps and eliminates the unevenness of the photodiode responses, defective

transmission from the optical system and the unvenness of the spectral source by carrying out a delayed homogenization. The analyser compares favourably with commercially available instruments with a single detector [74].

S
F$_1$
M$_1$
T
M$_2$
DIFFRACTION
GRATING
F$_2$
L$_1$
D
L$_2$ $\mu$.Ph
DEWAR
SYSTEM FOR DATA ACQUISITION AND TREATMENT
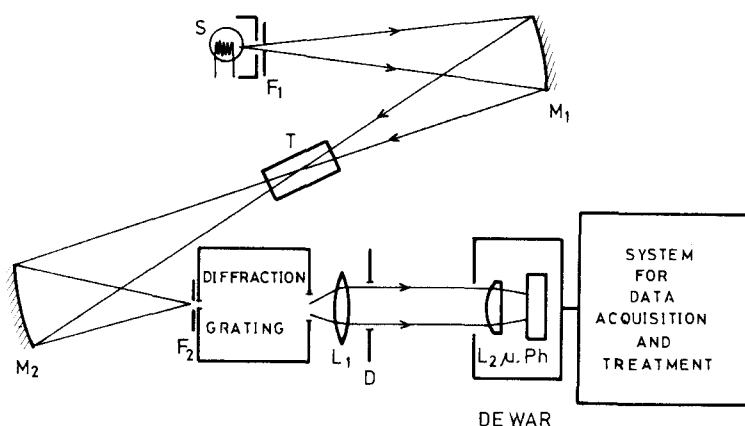
**Fig. 15.20** Experimental set-up for determination of NO$_2$ by means of a photodiode system and dispersive spectroscopy with computerized data acquisition and treatment. (Reproduced from [74] wih permission of the Natural Research Council of Canada).

Chemiluminescence techniques have been used for the determination of a variety or atmospheric pollutants [75], particularly ozone. Most the commercial instruments available for the determination of O$_3$ are based on chemiluminscent reactions and use the Nederbragt *et al.* method [76], which utilizes the light emitted upon reaction of ozone with ethylene gas (Meloy OA 325-2R and OA 350-2R ozone analysers) or that between O$_3$ and Rhodamine B over silica gel [77,78] or a disc with this reagent and gallic acid (Philips PW 9771 O$_3$ Monitor). Ray *et al.* have developed a chemiluminescence analyser for the measurement of atmospheric ozone using Eosin Y in ethylene glycol (Fig. 15.21). The sample gas is drawn through the detection cell at 1-7 L/min by a diaphragm pump. Fluid and air are separated at the reservoir, and the dye solution is recirculated. The sample gas flows across a paper or glass-fibre pad mat that is saturated with the organic dye dissolved in an alcohol solvent. The cell design allows the separate entry of air and dye solution through the upper inlet and their joint evacuation through the lower outlet. The response of the instrument, which compares favourably with that of commercial instruments (e.g. TECO Model 48P) is linear in the range 0.2-400 ppb [79].
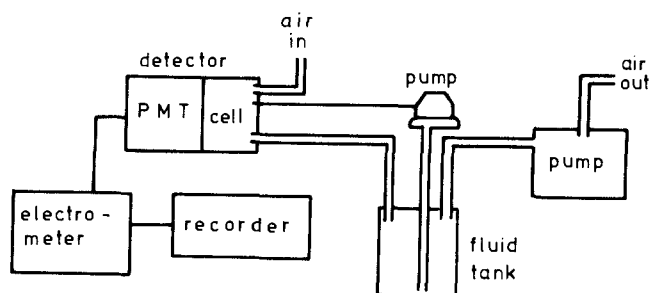
**Fig. 15.21** Scheme of chemiluminscence ozone analyser. PTM: photo-multiplier tube. (Reproduced from [79] with permission of the American Chemical Society).

The determination of hydrogen peroxide in the atmosphere has attracted much interest since it was acknowledged to promote the oxidation processes converting $SO_2$ to $H_2SO_4$ in the clouds below pH 4.5. The chief source of $H_2O_2$ dissolved in the clouds is that formed photochemically in the atmosphere. Vapour $H_2O_2$ concentrations in the range 4 pptv to 6 ppbv can be determined by an automatic fluorimetric method developed by Lazrus *et al.* [80] and based on the selective peroxidase-catalysed decomposition of $H_2O_2$ by (p-hydroxyphenyl) acetic acid (POPHA), which yields a dimer of the latter that absorbs at 320 nm and emits at 400 nm. Peroxidase also catalyses the formation of fluorescent dimers by hydroperoxides, so that in order to be able to distinguish $H_2O_2$ from organic peroxides a dual-channel flow system with a dual-cell fluorimeter is used. The above-mentioned reaction yields a measure of all peroxides in one channel, while the other is treated with the enzyme catalase, which destroys $H_2O_2$ selectively prior to the catalysed oxidation, so that the second channel provides an analytical blank for measurement of the $H_2O_2$.

An example of an automatic analyser for the determination of organic pollutants is the Meloy HC 500-2c from Columbia Scientific Industries. It is a self-contained system for monitoring ambient concentrations of non-methane hydrocarbons (NMHC), methane and total hydrocarbons. Sample air is first introduced directly into the flame ionization detector to yield a total hydrocarbon reading which is stored in an electrical circuit. The pneumatic system is automatically switched so that the sample air passes through a catalytic converter before it is introduced into the detector, which converts all the NMHC into a non-detectable species. Hence, only the methane in the sample is

sensed and its reading is stored in another electrical holding circuit. A differential amplifier subtracts the methane reading from the total hydrocarbon reading to provide an NMHC reading as the output signal.

Automatic dust meters are essentially based on two principles: light scattering and adsorption. Beta absorption is probably the most reliable means of making measurements insofar as these are independent of the phyiscal, chemical and optical properties of the monitored dust. Such is the technique used in the PW 9790 Dust Monitor from the Philips Environmental Protection Series. The instrument consists of three major parts: the measuring and control units and the vacuum pump. The measuring unit, shown in Fig. 15.22, consists of a filter tape drive system, a beta source and detector and a heated air sampling gate. Heating the air sampling gate eliminates condensation on the filter tape. Air is drawn through the section of the filter in the sampling gate by the vacuum pump. As the flow-rate of the pump is kept constant, by operating the pump for a preset time the volume of the air sample is accurately determined. The control unit regulates the overall timing of the dust monitor. It also contains the electronics used for the up-down counter, for derivation of output signals and for generation of the required d.c. power supplies. Plug-in wire links on one of the printed circuit boards enable a wide choice of counting (10 s to 4 min) and sampling periods (10 s to 14 h) to be preset.
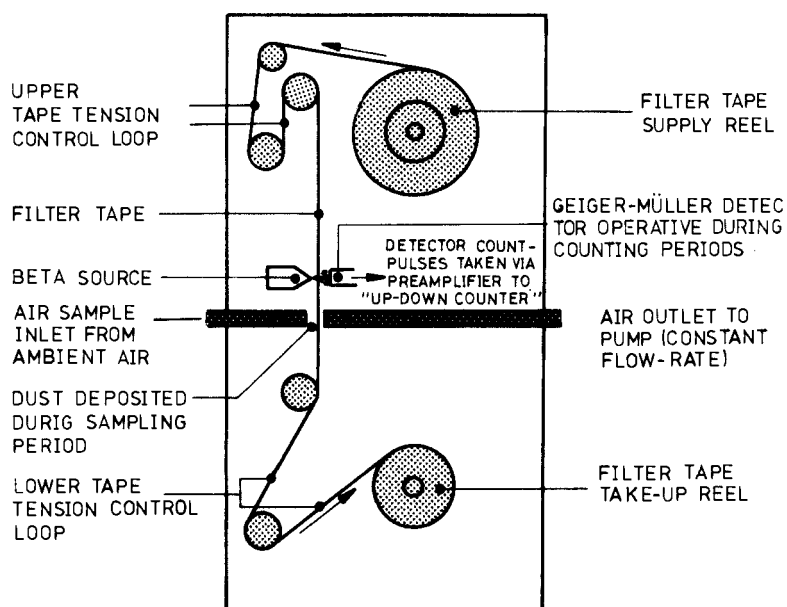


**Fig. 15.22** Physical arrangement of the Philips PW 9770 Dust Monitor. (Courtesy of Philips).

Radiation from a low-level beta source is passed through the clean glass-fibre filter and measured with a Geiger–Müller detector. The output pulses of the detector are counted by an electronic up–down counter over a preset counting period. A known volume of air is then passed through the same section of the filter, upon which dust from the air is deposited. The filter is exposed to the beta radiation, but this time the output pulses of the detector are used to count down from the number attained with the clean tape towards zero. Because of the dust film on the filter, however, more of the radiation is absorbed and the count rate is lower than before. The time taken to count down to zero is therefore longer than the preset counting period. The difference in time between the up and down-counting periods, which is accurately measured, bears a direct relationship to the mass of dust and, as the volume of the air sample is known, the result can be expressed as a concentration ($\mu g/m^3$). No repetitive calibration is required because the long-term drift is compensated by setting the zero level for each measurement —the zero level is equivalent to the beta count achieved through the clean tape— and the short-term drift occurring during the sampling period is negligible thanks to the extremely high stability of the instrument. The dust monitor can be operated continuously or started by remote control. The output signal is also suitable for on-line transmission to a remote data-handling centre. Alternatively, measurements can be logged on-site by using a recorder connected to the recorder output, or the analogue or digital signals can be used when the results are to be transmitted over a distance. The dust monitor can be used for more than measuring dust concentrations. As the filter collects actual dust samples, further studies are possible for completion, colour, shape, etc. An X-ray fluorescence spectrometer, for instance, can provide an elemental analysis of the sample for lead and iron.

### 15.4.2.2 Multi-parameter analysers

There are many instances in which the control of the composition of the atmosphere or that of gases dumped into it requires the automatic monitoring of more than one parameter. Multi-parameter air analysers can be classified according to the characteristics of the analytes to be determined into: (a) those in which only one product need be monitored (e.g. nitrogen oxides analyser) thanks to the interconvertibility of the sample components; (b) those using a single detection system responding to two different species (e.g. IR analyser for the determination of CO and $CO_2$); (c) those requiring a detector per analyte to be determined —these generally consist of a set of single-parameter analysers or of single- and multi-parameter analysers used in conjunction.

The functional principle behind nitrogen oxides analysers is generally the reaction of nitric oxide with ozone to yield excited nitrogen dioxide and water. The transition of the excited molecule to the ground state is accompanied by the release of radiant energy, which is suitably measured:

$$NO + O_3 \rightarrow NO_2^* + O_2$$
$$NO_2^* \rightarrow NO_2 + h\nu$$
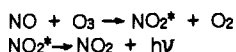
The radiation intensity is proportional to the rate of mass flow of NO in the reaction chamber, where it is mixed with ozone. The radiant energy is converted to an electric output by means of a photomultiplier tube and its associated electronics. A converter transforms the $NO_2$ initially present in the sample to NO, so that, by means of a solenoid valve, the incoming air sample is alternately passed through a converter by-pass to sense NO alone and through the converter to detect the sum of NO and $NO_2$, defined as $NO_x$. An electric subtraction circuit provides the $NO_2$ concentration as the difference between $NO_x$ and NO. Figure 15.23 shows a block diagram of an analyser of this type, namely the Model 1600 $NO/NO_2/NO_x$ Analyser from Columbia Scientific Industries. As can be seen on the left, the instrument consists of an ozone generator and a reaction chamber where the $O_3$ is mixed with the sample, which is passed or not through the oxide converter through the action of the motorized three-way valve. The right-hand side of Fig. 15.23 depicts the sensing, discrimination and data delivery systems, namely an optical filter collecting the emission from the reaction chamber, a photomultiplier tube (PT) and an electronic circuit that allows the arrival at each recorder and/or microcomputer of the signal corresponding to the sum of the concentrations of the analytes or of each of them separately. The panel meter can display the value of any of the three signals without the need to interrupt the instrument's operation. The three-way valve works at a rate of 480 cycles/h, which substancially reduces the errors associated with the rapid change in the $NO_x$ levels in the surrounding environment. Gas flow-rates are kept within $\pm 1\%$ of the initial setting by means of orifices kept at $50°C$ and protected by stainless $7-\mu m$ steel filters. The photomultplier photocathode is kept refrigerated at $10\pm0.1°C$ to reduce background noise and achieve a detection limit of 0.002 ppm. In order to avoid the contribution of ammonia to the $NO_x$ measurement, the converter operates below $400°C$. The chemical/catalyst cartridge is designed so that the air sample must pass through a packed column to ensure intimate contact between the air and the catalyst, and so guarantee 99% conversion.

Infrared spectrometers are the best alternative to the continuous monitoring of CO and $CO_2$. An autoanalyser of this type is depicted schematically in
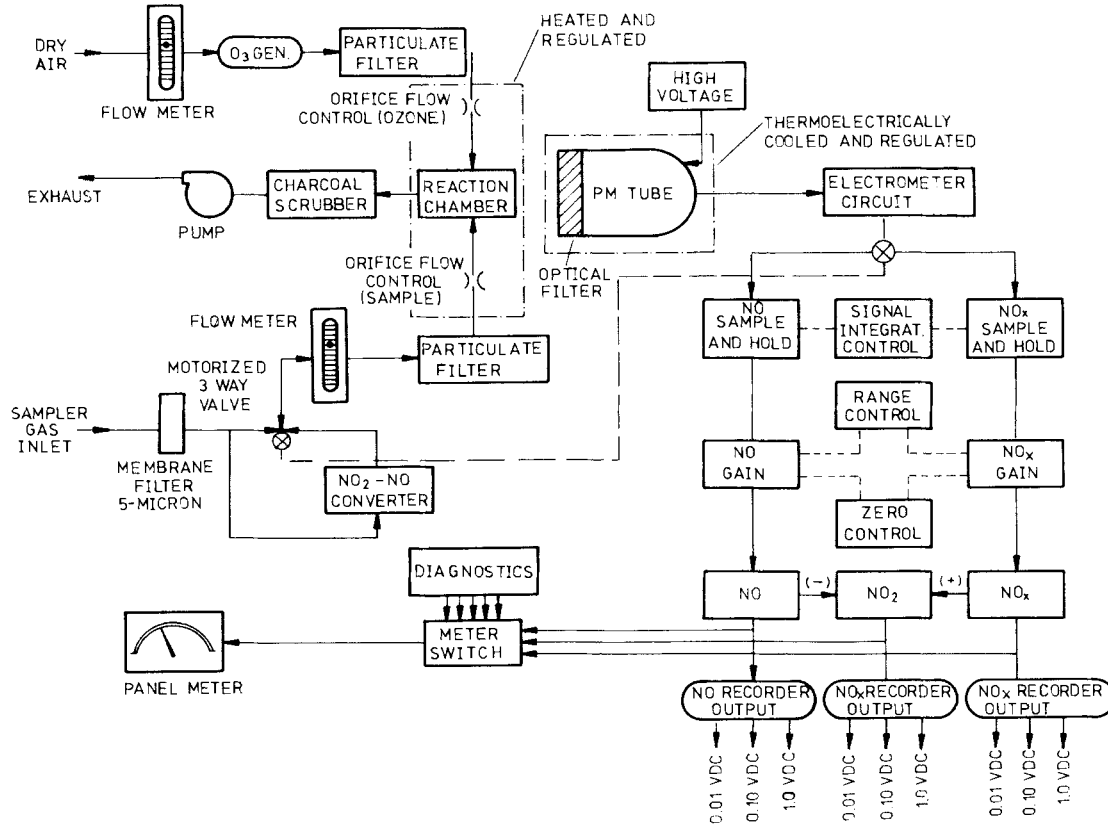
**Fig. 15.23** Block diagram of nitrogen oxides analyser. (Courtesy of Columbia Scientific Industries Corporation).

Fig. 15.24a. A portion of the gas from the atmosphere or an industrial chim-
ney is sucked by a pump and passed through a filter with a pore size ensuring
retention of unwanted particles —this is the sampling step, which may include
the measurement of the moisture in the collected gas. In the measuring stage,
the sample is passed through a solenoid valve, a pressure gauge and the IR
analyser, which measures the absorption of both analytes at their character-
istic wavelengths. The data generated and the sample pressure are acquired by
recorders and/or a computer which allows the determination of the atmospheric
concentration of both analytes. The solenoid valve allows the arrival of CO
and $CO_2$ standards at the detector to run the pertinent calibrations.

The joint use of single- and multi-parameter analysers permits the moni-
toring of a variety of analytes. The instrument depicted in Fig. 15.24b is
used for monitoring blast furnace processes. Dirty gas is extracted from the
duct and pulled through the filter by a pump. A small portion of the clean wet
gas after the pump is fed to the moisture analyser, but the major portion is
cooled in a refrigerator to remove the bulk of the moisture before it is
pumped to the analysers. The relatively dry gas flows approximately 150 ft
from the pump shed to the analyser room. By means of a by-pass valve the sec-
ond pump boosts the gas pressure sufficiently to force the gas through a final
drier and into the analyser. A major portion of the gas extracted from the
plant duct is passed through the by-pass valve. Only a small portion of the
total volume is used for analysis. A large volume of gas is pumped to decrease
the residence time in the cleaning system because the analysers are located at
a distance of about 300 ft from the plant duct. The volume of the gas appor-
tioned for analysis is dried to a moisture concentration of a few ppm. A solen-
oid valve system automatically or manually allows the selection of blast
furnace gas or any of three calibrating gases to flow through the pressure
controller and into the analysers. All the gas flows through the hydrogen
cell, the $CO_2$ cell and, finally, the CO cell before it is vented to the
atmosphere. Signal lines are connected from each analyser to the respective
recorders, which are equipped with transmitting slide wires that furnish a
signal to the computer terminal and then to the main computer. With few ex-
ceptions, a well-designed analyser system meets the requirements of several
processes. However, a sampling system is not predictable. Although the same
raw material is used, the particulate matter in the gas from one furnace may
vary significantly enough from the dust in a second furnace to require re-
design of the filtering media. The difference is not in the amount of dust,
but in its composition and physical characteristics.

Figure 15.24c shows the scheme of an analyser for basic oxygen processes
(BOP). The gas flows from the plant duct through the filters to the pump.
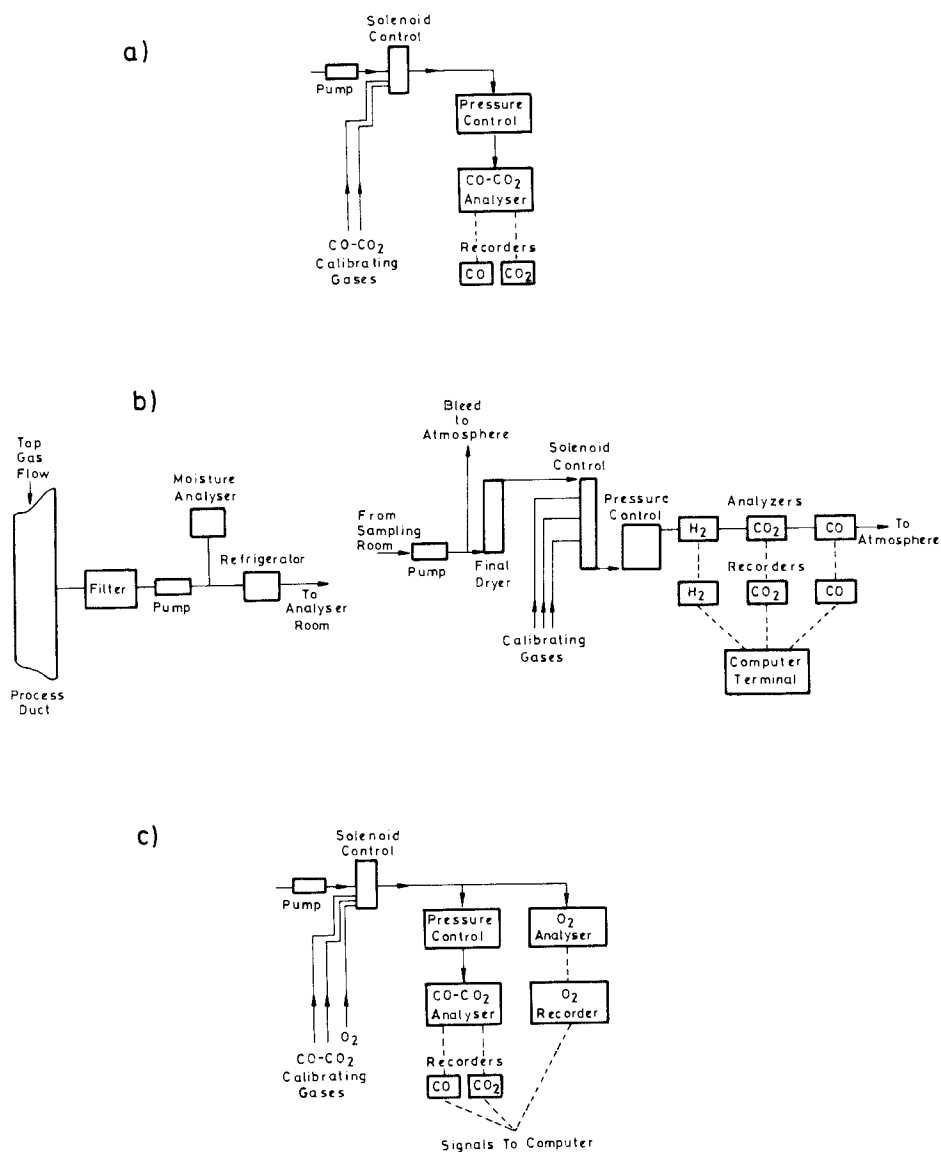
**Fig. 15.24** (a) Analyser with IR detector for the determination of CO and $CO_2$. Analyser with single- and multi-parameter detectors for blast furnace (b) and basic oxygen process (c). (Reproduced with permission of Academic Press).

After the pump, a portion of the clean wet gas is diverted to the moisture analyser. The major portion flows through a refrigerator to remove moisture to approximately 0.8% V/V. The gas pressure is boosted by a pump and diverted to the paramagnetic oxygen analyser and the $CO_2$ infrared analyser. If CO is also present in the process gas, both CO and $CO_2$ cells are included in the same analyser cabinet. The recorders are equipped with transmitting wires which furnish signals to the computer located approximately 500 ft from the analysers. A response time of 20 s is critical in the BOP process, so that the sampler must be located in the dirty gas stream after the first thorough mixing. The composition and physical characteristics of the dust particles differ considerably from those in the blast furnace. The selection of filter media is based on the dust characteristics. The analysers can be calibrated manually or automatically. The CO and $CO_2$ analysers are standardized at two points on the concentration curve, one in the neighbourhood of the low end and another in that of the maximum concentration in the process gas. The $O_2$ analyser is also checked at the high and low ends with calibrating gases.

Analyses for pollutants in open atmospheres are more useful on account of their versatility, the use of single-parameter modules or analysers making up multi-component monitoring stations adaptable to the requirements of the area to be monitored. A typical scheme of one such station, namely a multi-component monitoring system marketed by Philips, is depicted in Fig. 15.25. The operational principle behind each of the measuring systems (coulometry for $SO_2$, $H_2S$, NO and $NO_2$, and chemiluminescence for $O_3$) was dealt with in describing single-parameter analysers. A pollutant analyser performs the functions of sampling, analysis and calibration. The actual pollutant analysis and calibration take place in the monitor. This contains the essential items such as filters, standard source, valve (which controls the zero-checking, span-setting and measurement sequences), detector and air-flow controller. The supply cabinet houses the air suction pump (suitable for use with up to three monitors except for the $O_3$ monitor) and a pre-wired, three-position module rack. Each position can accommodate one plug-in, electrical supply unit, and the number of supply units fitted (up to three) depends on the number of monitors used. The remaining module positions can be fitted with accessories such as a transmission frame (two positions) or a control clock (one position). The air sampling, can be carried out in two ways: (a) by means of a sampler with a built-in dust filter which can serve up to three monitors depending on the ambient dust concentration in the atmosphere, and (b) by use of a multi-component monitoring station, often much better for drawing a large air sample through a large-bore pipe. Both methods ensure the removal of water, insects and large dust particles from the sampled air. Regarding data transmission, in
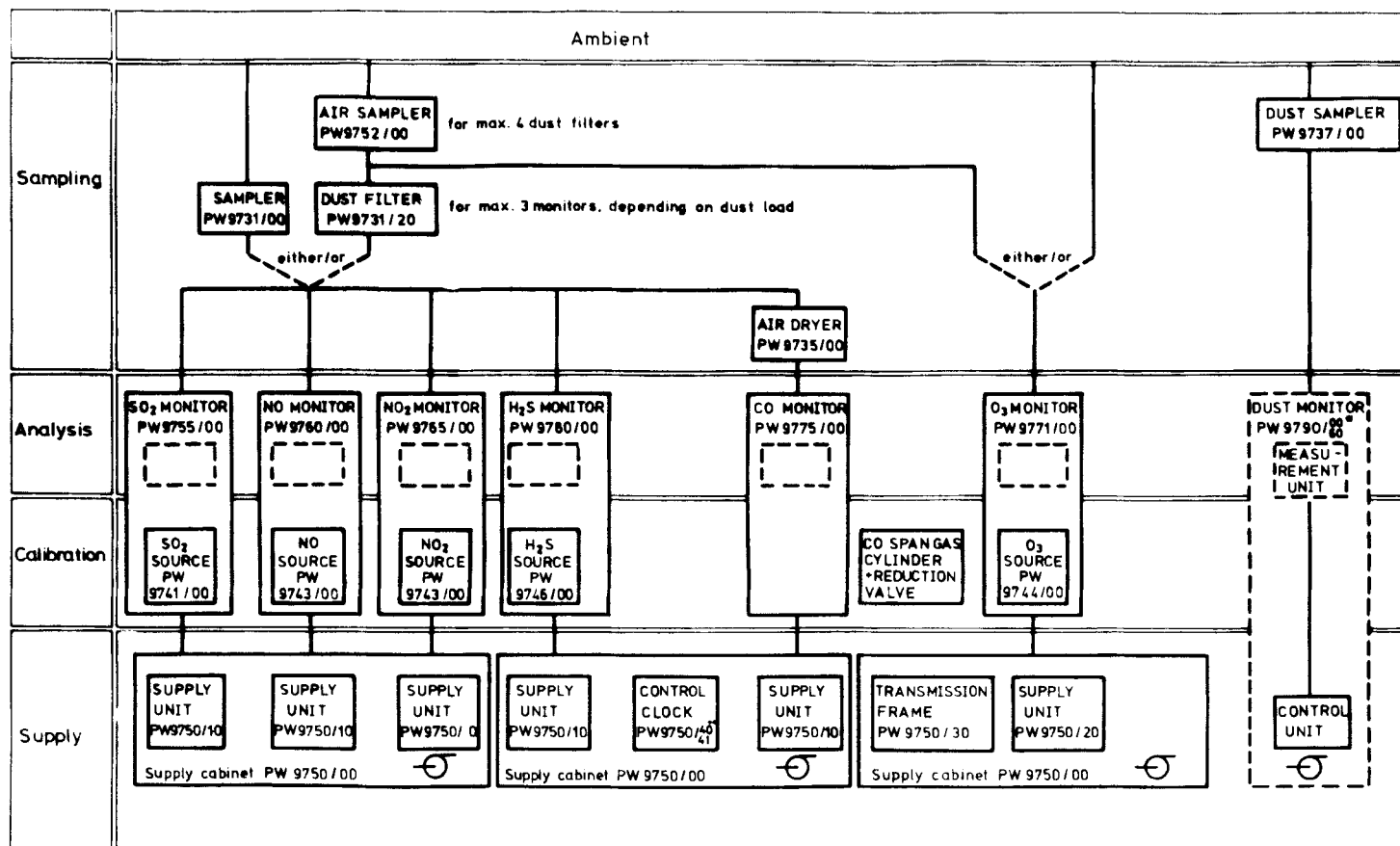
**Fig. 15.25** Scheme of a multi-component air pollution monitoring system. (Courtesy of Philips).

a simple installation where a visual indication of the analysis is sufficient, the direct output signal of the monitor can be logged by a recorder. When it is necessary, however, to transmit the output signal to a remote data-handling centre, the signal must be converted into a form suitable for transmission over switched or leased telephone wires, or via radio links —see the next section.

## 15.5 DATA ACQUISITION, TRANSMISSION AND PROCESSING. SURVEY NETWORKS

The need for the rapid acquisition of the results of an environmental ana- lysis is of outmost importance insofar as such results may indicate the need to take drastic and urgent steps in cases where some danger or damage may be otherwise expected. Hence, the rapid acquisition of data by means of an A/D converter is virtually indispensable in environmental analyses to complete the achievements of automatic sampling and analysis [81–84] —the automatic sam- pling of pollutants in water and air is beyond the already broad scope of this chapter [47]. An instrument specially designed for this purpose is the micro- processor-based Pollution Data-Reductor (micro-PDR) from Philips, conceived for fully automatic pollution monitoring and providing a straightforward and
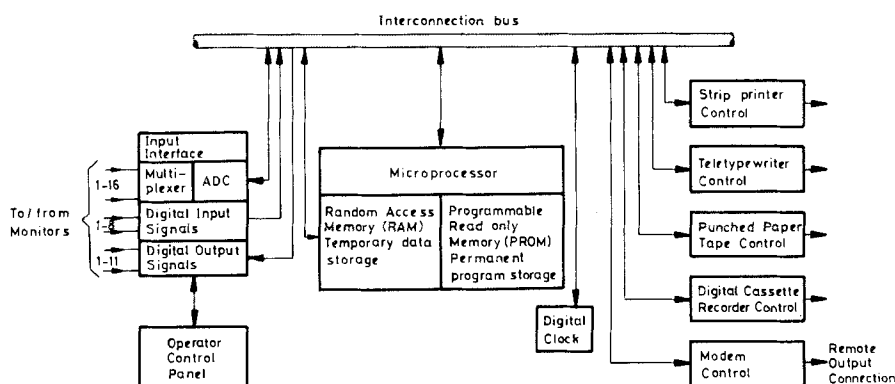


**Fig. 15.26** Block diagram of a microprocessor-based pollution data re- ductor. (Courtesy of Philips).

efficient means of automatically acquiring, processing and recording the data from a series of pollution monitors. In addition, the system can start calibration cycles and use the results to correct the data from the analysis of the samples. The output data can be recorded in a variety of supports: magnetic tape cassettes, numeric or alphanumeric print-outs and punched paper tape. Remote printing and connection to a larger processing system are also possible. A block diagram of the micro-PDR is shown in Fig. 15.26. The primary functions of the system are data acquisition and processing, output of control signals to monitors, timing and data output.

*Data collection.* Analogue input signals (0–5 V or 0–20 mA) are received either direct from a local monitor or via the supervision multi-tonal (MDF) equipment when coming from remote monitors. The data inputs are switched serially by the solid-state multiplexer, under the control of the microprocessor, to the A/D converter. Here, the analogue signals are converted to digital form and then taken, together with up to eight digital signals, via an interconnection bus to the microprocessor and the temporary data store (the RAM). All monitors are sampled once per minute.

*Data processing.* The microprocessor functions according to the program stored in the programmable read-only memory (PROM), a solid-state data-storage device whose contents is virtually indestructible. The main tasks of the 8-bit microprocessor are: (a) control of the input multiplexer; (b) start and control of the calibration cycle; (c) correction of measured values according to the results of the calibration cycle; (d) computation of periodic averages; (e) calculation of output data; (f) output of data and (g) control of peripherals.

*Output of control signals to the monitor.* Up to three control signals can be sent to the pollution monitors via the digital output control. One of these signals is the calibration cycle control signal.

*Timing.* An accurate, real-time digital clock is used for timing all events. At the heart of the digital clock is a crystal whose output is used to derive the minute, hour and day signals. From these, the microprocessor obtains the months and years.

*Data output.* Ouput information is taken via appropriate control units to the respective output peripherals, as shown in the block diagram in Fig. 15.26. The modem output can be used either to connect a remote teletypewriter to the micro-PDR, or to connect this to a central station. These remote connections are achieved over either leased or switched telephone lines (dialled link). During normal automatic operation, the following output data are registered: (1) station identification, (2) status message, (3) date and time, (4) 1/2-h average, (5) 24-h average, (6) all data or functions manually entered into the system.

   *Protection against loss of data due to power failure.* The microprocessor and digital clock are provided with reserve power supplies, which ensures that, if the normal mains supply should fail, the timing continues uninterrupted and there is no loss of data from the RAM. After restoration of the normal supply following a power failure, the time, date and stored data are recorded by the output equipment and normal automatic operation is resumed. Recharging of the reserve power-pack also takes place when the operation from the mains supply is restored. No reserve supply is required by the PROM, whose memory contents are unaffected by disturbances to the mains.

   The most complex fashion of performing environmental sampling, analysis and control is probably that used by automatic water and atmospheric pollution survey networks. A survey network consists of a series of stations, each of which has a modular multi-parameter analyser transmitting data to and receiving commands from a central computer that controls and organizes all the stations making up the network. The scheme of one such network for water control is depicted in Fig. 15.27.
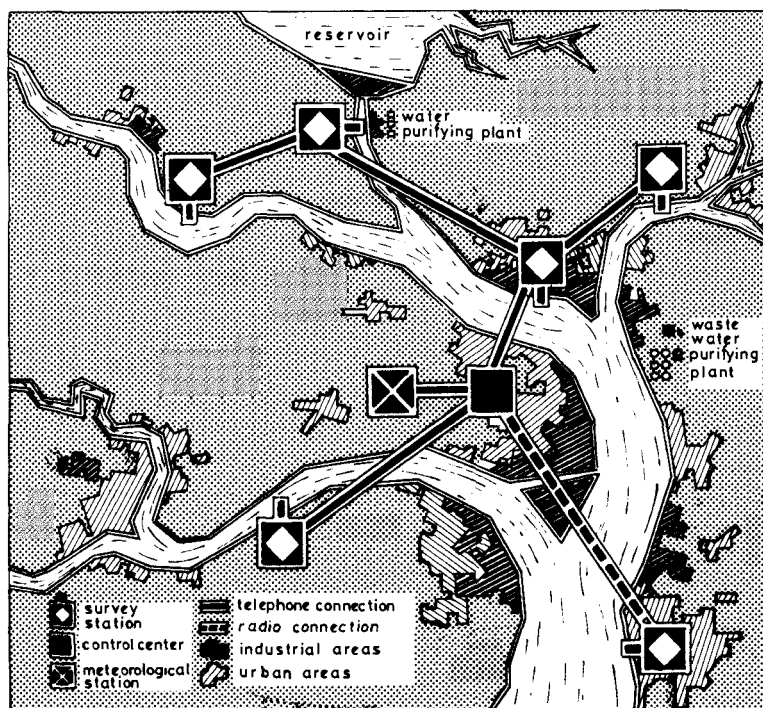


**Fig. 15.27**   Scheme of river water survey station. (Courtesy of Philips).

   The site of a survey station must be in accordance with the pursued objec-

tives: in the case of water survey, the site should be as close as possible to the drainage point, where collected samples will be more representative than those taken at points where the waste waters would have merged throroughly with those of the river and the results obtained would be representative of the water quality of the river itself. Dual sampling can be carried out at the confluence of two water-ways to separately measure, record and transmit the data corresponding to both.

The overall functioning of a survey station involves three basic operations: data transmission and communication, data reduction and data recording. Data transmission by means of various systems such as the multitonal survey system (MDF) allows signals to be sent in both directions between the stations and the central unit by means of private or hired telephone lines. A single telephone lines enables transmission of up to 25 data channels according to the recommendations from CCITT. Digital transmission (MDT), based on the time-division multiplex principle, can prove economical in sending large volumes of measurement, status or remote control signals, which must be converted to digital form prior to transmission. Switched telephone lines shared with other users are of use when great distances are to be covered and hired lines are too expensive. In this case, stations require a self-contained control and memory system to collect and store information. This "intelligent station" device sends information to the pollution data reductor in the network centre when requested by this, so that the telephone line is not continuously engaged. Normal transmission/reception units (radio links operating in the VHF or UHF bands) can also be used for this purpose. This method allows up to 16 survey stations to be linked to the data reductor on the same carrier frequency, which is accomplished by using a simplex radio-link with selective calls to each station in the network.

The so-called 'pollution data reductor' (PDR) is the unit where data are processed. Depending on the size and purpose of the network, it can be as simple as an analogue tape recorder or as complicated as a programmable computer with various output readouts and the capability to carry out alarm functions in real time. This information can be used to warn those industries causing pollution or being on the verge of suffering its consequences. The PDR can be of one of two types: fixed-program (without computer) and stored-program (with computer). Fixed-program PDRs are chiefly used in uncomplicated networks whose principal aim is to record data, whether instant concentrations in analogue form or average concentrations in digital form. Stored-program PDRs are employed when on-line calculation and recording are required. The typical functions performed by this processing system are as follows: (a) estimation of short-term pollution predictions; (b) checking of the functioning of the sta-

tions, transmission network and PDR; (c) recording of data on punched paper or magnetic tape for batch processes; (d) data concentration and line connection to a central pollution processor (CPP). The key unit in a stored-program PDR is the programmable mini-processor, whose software consists of (a) a monitor program running in real time and governing the remainder of the programs, equipment and peripherals; (b) a program for handling of messages (errors, warnings, recordings of pollution data); (c) a program controlling data acqui-sition throughout the network every minute, hour, fraction of hour or day; (d) a calibration program controlling the calibration cycle and the interpretation of the calibration data; (e) a keyboard function program controlling the com-munication between the user and the machine, and between the control centre and the stations in the network.

As a rule, it is more convenient to transmit measurements to a control centre, laboratory or office, where they can be immediately read from the tele-typewriter, from punched paper or magnetic tape. The on-site recording of data in the station is also of use for field research, although more limited as re-gards long-term information. A data reductor can also be installed in the sta-tion or be connected to it by remote control for averaging of some parameters (recording of integrated values).

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# 16
## Process analysers

### 16.1 INTRODUCTION

Process analysers are automatic devices adapted to their environment and conceived for the continuous or periodical measurement of one or more physical or chemical parameters along an industrial process line. In addition, they deliver results in a suitable manner and allow for correction of the operational conditions of the process on the basis of such results. They are automatic insofar as they require no human intervention and must be adapted to their environment because of the generally adverse environments prevailing in industry. Results must be delivered in the appropriate form and units. There are still relatively few analysers capable of taking direct and active part in the industrial process which they are meant to control; however, the gradual incorporation of microprocessors will no doubt endow process analysers with greater reliability, which will result in higher degrees of automation at lower cost.

Until the recent advent of robot stations, analysers furnished with samplers and systems for data treatment and output represented the highest degree of automation, even though the operator treated the sample and placed it in the analyser. Industrial process analysers are generally equipped with sampling systems that prepare the sample and transfer it under the optimum conditions and in the shortest time possible to the analyser. In addition to performing the analyses, a few analysers are programmed to make decisions about the operational conditions suited to the process line according to the results obtained. This is therefore one more step in the automation of the analytical process, imposed by necessity rather than by a desire to increase the sophistication of analysers. In fact, some industries involve environmental conditions resulting in hazardous sample collection and treatment which are best left to a process analyser for safety reasons; others are better off by devoting the personnel required for sample collection, transfer and treatment to other tasks. Another strong reason for the use of these analysers is the need for rapid analyses. Hence, the term 'analyser' is more accurate here than other alternatives for referring to these instruments insofar as they take part in all three stages of the analytical process (see Chapter 1).

Process analysers are *continuous* in nature (see Chapter 1), i.e. they continuously extract sample from the line of the process they control; there is no physical separation between samples in individual containers. In addition, the analyses they carry out can be *continuous* when the sample is monitored uninterruptedly (e.g. infrared analysers, conductimetric and hygrometric probes) or *periodic* when the sample is analysed at preset intervals (e.g. gas and liquid chromatographs).

## 16.2 FEATURES OF PROCESS ANALYSERS
### 16.2.1 The optimum analyser

There are more marked differences between process analysers than there are between laboratory analysers owing to the greater variety of requirements and experimental conditions involved in different industries. Despite their diversity, every process analyser should have a number of desirable features, namely:

(a) The chemical method applied should be as simple as possible with regard to the number of reagents, diluents, transfer gases, reference fluids and temperature and pressure conditions used to reduce control and maintenance to a minimum. In addition, accessible and affordable reagents rather than special, high-purity chemicals should be used. Methods involving excessive corrosion should be avoided at any rate.

(b) Continuous analysers are generally to be preferred to their batch counterparts because the greater the number of different operations involved in the analyses, the lower is the reliability of the analyser.

(c) Measurements should be sufficiently accurate and precise to ensure efficient control of the process line. In addition, the system should carry out the analyses and deliver results in the shortest possible time in order not to alter the sample and to facilitate rapid correction of the operational conditions if the results make it advisable.

(d) The analyser should be easy to operate by relatively unskilled workers. It should require no special attention or maintenance for long periods so as to save time and money. The instrumental design should facilitate its maintenance without disturbance to the process line. Ideally, the instrument should be capable of performing autocalibrations or require only occasional adjustment.

(e) The system should have the means to prevent deterioration of and keep dirt from the sensing element resulting from the occurrence of suspended solids or chemical attack. This will result in a longer analyser lifetime and reduce maintenance. Moreover, the analyser should be protected from its en-

vironment and the reagents used in order to avoid mechanical failures that might have drastic consequences.

(f) Process analysers devoted to control industrial safety or waste need no economic justification as they are imposed by current laws. However, analysers meant to control industrial process lines should be purchased with all the above requirements plus their economy in mind. This involves carrying out an economic study, weighing production (or sales) increases against different factors such as purchasing, installation and operating costs (depreciation, maintenance and utilities) [1].

In practice, process analysers do not have all of the above desirable features; instead, they are a compromise between the requirements of the process to be controlled and affordability.

### 16.2.2 Relationship between process and laboratory analysers

With few exceptions, the development of process analysers is one generation behind that of laboratory analysers. It is significant that many process plants are still controlled by pressure, temperature, flow or fluid level meters, easy to instal, calibrate and maintain.

There are various reasons accounting for the long delay involved in the adaptation of well-known laboratory techniques to the control of industrial processes:

(a) The conservativism of employers and technicians, who generally are reluctant to change elementary techniques that provide acceptable results by new analytical techniques.

(b) The physicists and chemists who develop new laboratory techniques are not concerned with their application to industrial processes. This is rather a task for engineers, who are aware of the needs involved and difficulties posed by industrial plants. Unfortunately, there are many fewer engineers interested in process analysis than physicists and chemists doing laboratory research.

(c) An appropriate system for sample collection and conditioning allowing the extraction of a representative sample from the process line and its transfer to the analyser in a short time and without significant alterations is as vital as or even more so than the analyser itself, so much so that such a sampling system is sometimes more expensive than the analyser proper.

(d) A process analyser controlling the inflow of raw materials in an industrial plant can save large sums of money if it works properly; otherwise it can result in economic losses putting the viability of the plant at stake. However, errors in the functioning of analysers controlling the level of toxic species in the atmosphere of an industrial plant may even give rise to the loss of human lives. This responsibility seldom affects manufacturers of labor-

atory analysers. This is therefore one of the reasons accounting for the conservatism of process analyser manufacturers and the long study of and experimentation with pilot plants needed before an analyser prototype is launched with the expected safety and reliability warranties.

### 16.2.3 Laboratory analysers adapted to industrial processes

Occasionally, process analysers are chosen for a given type of analysis on the grounds of its proven efficiency in the laboratory rather than its suitability for the process in question. This reasoning is unwise because the fact that a given method or technique is suited to laboratory requirements does not necessarily imply that it is fit for application to an industrial process on account of the differences between both extremes. In fact, there are four basic differences between laboratory and industrial conditions, namely:

(a) *In the purpose.* Laboratory analysers are designed to be flexible, both in the species that can be analysed and in the operational conditions, which makes them more complex and delicate than process analysers. These are conceived to perform a specific analysis under clearly established experimental conditions and are therefore simpler, cheaper and more solid.

(b) *In the performance.* Process analysers are expected to have longer times than laboratory analysers as they work non-stop 24 h a day, 7 days a week instead of periodically 8 h a day. Thus, a laboratory HPLC column is no longer reliable after *ca.* 1000 injections —a few months for a laboratory but as little as 1 week in an industrial plant, which will therefore require columns lasting at least ten times longer.

Laboratory instruments are usually well attended to. Their operation and maintenance are entrusted to skilled personnel. They are calibrated prior to each series of analyses and are cleaned thoroughly afterwards. Process analysers should not require much attention as this detracts from economy: the personnel in charge of the analyser are generally unskilled and have a secondary interest in it —they are rather concerned with the process line. Maintenance should be kept to a minimum and the instrument should be designed so as to avoid drifts resulting in the need for frequent calibration or, alternatively, be equipped with an autocalibration mechanism.

(c) *In the accessory elements.* As stated above, process analysers have automatic systems for sample collection and conditioning, allowing the sample to be transferred from the analyser without human intervention and be treated appropriately to make it as little corrosive to the analyser as possible in order to ensure the reliability of the analyses. These sampling systems are often more complex and expensive than the analysers themselves. As is widely

known, most laboratory processes involve the manual treatment of the sample prior to its manual or automatic introduction into the analyser. On the other hand, process analysers must include a means of transmitting signals from the analyser site to the control room of the industrial plant, which often involves selection of the appropriate lines and interfaces, protection of the transmission lines from the effect of electromagnetic radiation and voltage spikes, in addition to grounding. Laboratory instruments do not require this ancillary equipment since the analyst and the analyser are usually in the same room and complement each other: the analyst places the samples in the analyser and this provides the former with the results. Finally, it should be emphasized that process analysers usually operate under extreme temperature, humidity and corrosion conditions, in contrast to laboratory instruments, whose working environment is much safer. Hence they require special protection against aggressive agents and therefore conditioning of potentially hazardous samples, in addition to appropriate construction materials and the installation of malfunction alarms. Moreover, they must comply with strict safety rules of Class 1, groups C and D, divisions 1 and 2 of the National Electrical Code [2].

(d) *In the manufacture.* The liability of manufacturers of process analysers is much greater than that of laboratory analyser manufacturers. This is understandable taking into account that the instrument malfunction may result in a build-up of unsatisfactory product or in the unjustified halting of the process with resultant economic losses. Process analysers must therefore be designed to offer the maximum security to the potential purchaser. On the other hand, laboratory analysers are sold in isolation, with no further compromise on the part of the manufacturer; yet, whoever buys a process analyser also purchases the engineering study required to perform a specific analysis on a given process line.

### 16.2.4 Advantages and disadvantages of process analysers

Because of the differences between laboratory and process analysers, the latter are better suited to the control of process lines, a field in which they possess they following advantages over the former:

(a) *Rapidity.* Because of the closeness between the analyser and the process line, analyses are carried out much faster than with laboratory analysers, usually located at some distance from the process line. This results in five significant advantages:

– The possibility of performing fast analyses allows the chemical engineer to introduce appropriate changes in the operational conditions of the process line as soon as its optimum performance is altered.

- The quality of the resultant products is more uniform.

- Thanks to the rapidity with which analyses can be performed, unstable samples undergo no significant changes in composition, which is a great asset if samples must be transported to a distant laboratory.

- The time per analysis is shorter in process analysers, which are thus ideal whenever fast and frequent measurements are compelled by rapid and appreciable changes in the composition of the process stream.

- Transient changes in the process which otherwise would go unnoticed are readily detected.

- The analysis and control of the process line can be readily automated.

(b) *Economy.* The use of a process analyser also offers a series of savings, namely:

- In laboratory staff, who are not required.

- In cost per analysis, as a result of the high degree of automation.

- In energy consumption and hence in production costs.

- In the re-processing of materials not conforming to the specifications. Some other advantages are characteristic of particular areas such as the oil industry, where process analysers allow the lifetime of catalysts to be lengthened and coking and distillation flooding to be reduced.

(c) *Improved sampling* as a result of:

- The elimination of the sampling errors and labelling involved in human intervention.

- The need for no special containers for the analysis of gases.

- The capability to analyse toxic or radioactive samples that are hazardous to humans.

Laboratory analysers in turn have some advantages over process analysers. Thus, (a) they allow the analysis of solids or samples requiring complex pretreatments of difficult automation —this will no doubt be solved by the growing advances in robotics; (b) they require less protection from hazardous environments and allow for frequent calibration and maintenance; and (c) they are fit for the analysis of various samples from different process lines.


## 16.3 CLASSIFICATION OF PROCESS ANALYSERS

Because of the large variety of industries and the different needs of industrial plants, there is a vast range of process analysers in use, which can be classified according to different criteria, namely:

### 16.3.1 According to the location with respect to the process line

As pointed out by Frant and Oliver [3], process analysers can be classi-

fied into three types according to their location with respect to the process line: 'off-line', 'on-line' and 'in-line' in increasing order of closeness between the instrument and the process line. *Off-line* analysers are those to which samples extracted from the process lines are taken for analysis —their advantages and disadvantages were discussed in the preceding section. *In-line* analysers have their sensors inserted directly into the process line. Finally, *on-line* analysers are located close to the process line but do not have their sensors inserted into the process stream; instead, they have a system for collection and conditioning of samples —which are prepared not to damage the sensor and freed from interferences— and for transporting them to the analyser. However, non-invasive analysers are representative of current trends, although they are not yet commercially available [4]. Like in-line analysers, they are characterized by the fact that the analyte is determined without extracting the sample from the process line; however, at no time does the sensor come into contact with the sample as it does in the former, so that they have all the advantages of in-line analysers but none of their disadvantages.

The sole difference between on-line and in-line analysers lies in the location of the sensor, which involves different designs and suitability for different situations. In-line analysers are simpler than their on-line counterparts insofar as they require no sampling system for extraction of the sample from the process sream; it is rather the sensor which goes to the sample. Because of this, they can only be used to control process lines carrying components not liable to damage the sensor, interfering with the analysis or dirtying the sensor appreciably —otherwise they will require an automatic cleaning system; in addition, they should require little maintenance and recalibration as these involve stopping the process stream, which is a great disadvantage. Examples of this type of analyser are pH and dissolved oxygen meters, and conductimetric and hygroscopic probes. However, they are uncommon because the samples generally require conditioning prior to performing the measurements so that they are free from interferences and the analyser has to undergo as little sample attack as possible. For this reason, on-line analysers are commoner at present.

### 16.3.2 According to purpose

Depending on their objective, industrial analysers can be classified into *safety* or *protection* analysers, designed for the measurement of pollutants, and *production line controllers*, intended for the analysis for one or more components in a process line. The former in turn can be classified according to whether they are meant to control industrial waste (gases or liquid efflu-

ents) or the purity of the working atmosphere in an industrial plant. The use of these analysers is compelled by laws for protection of the environment and industrial safety and hygiene in those industries producing polluting species. Because of their very nature they require no economic justification and must be accurate, as their chief purpose is to determine the concentration of noxious species in the analysed fluid. They are therefore sophisticated and delicate instruments requiring highly specialized technical support and maintenance. Analysers devoted to the control of production lines can be classified into those determining the quality of the end product and those controlling one or more species representative of the efficiency of a process. Obviously they require an economic study of their viability to be performed prior to purchase. Those used for quality control should be as accurate as safety analysers, while those intended for the determination of process efficiency should be precise rather than accurate because the technicians controlling the process are often more interested in changes than in absolute values, as they usually correlate the instrument output with the process efficiency without bothering about the real meaning of the output.

### 16.3.3 According to the result interpreter

As stated above, some process analysers are almost completely automated as they have not only an automatic system for sample collection and treatment, but also a computer processing the data received from the analyser proper and acting on the process line according to a program; there is no human intervention in any stage of the measuring process, only in their maintenance. These, the so-called 'indirect control process analysers', are uncommon because of their high sophistication. Their 'direct' counterparts require no processing of their outputs, which are readily interpreted by a technician acting on the operating conditions of the process line depending on the results.

### 16.3.4 According to the type of parameter determined

Industrial analysers can control physical or chemical parameters. Physical parameter analysers (conductimeters, viscometers, refractometers, pressure and temperature meters) frequently measure and control only one property of the fluid, the variation of which generally depends on a single component that is controlled in an indirect fashion. Chemical parameter analysers directly measure the concentration of one or more species in a fluid. They can be specific for a given species (e.g. pH-meters, potentiometers with ion-selective electrodes, oxygen meters) or control several species simultaneously (e.g. gas or liquid chromatographs) or successively (photometers) with minimum alterations.

**16.4 COMPONENTS OF A PROCESS ANALYSER**

As stated above, there is a large variety of process analysers adapted to particular needs; however, most of them have the following elements in common: (a) a sampling system; (b) the analyser proper; (c) the result delivery system and (d) the analyser protection.

Experience has shown that the sampling system of a process analyser is as important as or even more so than the analyser itself. On the other hand, the analyser must be duly protected to ensure smooth functioning and a reasonably long lifetime with consequent reliability and profitability. Hence, both elements are commented on here in greater detail.

**16.4.1 Sampling system**

In practice, choosing the appropriate process analyser is only part of the problem faced when trying to perform analyses on a process line in a reliable manner; moreover, the selection of the analyser is greatly influenced by the possibility of obtaining a suitable sample. The conditioning of the extracted sample is generally of utmost importance, so much so that it has given rise to the assertion that "an analyser is as accurate as the sample it receives" (i.e. problems with the analysis are bound to arise from inappropriate samples rather than from malfunctioning of the analyser). For this reason, it is not unreasonable to spend as much or even more time and money on the design and construction of the sampling system than on the analyser itself. The design of the sampling system and selection of the analyser should therefore receive great attention in order to avoid unnecessary expense in terms of waste material, the client's dissatisfaction and maintenance problems. Thus, failures in a transfer analyser controlling the quality of massive-produced goods in a given species may result in enormous losses for the manufacturer or the purchaser; an analyser that fails to provide an accurate measure of the level of oxygen or a toxic species in an industrial plant may cause a tragedy involving the loss of human lives [5].

Process analysers, like computers, can only provide accurate results if they are fed with appropriate starting materials —samples and information. Sophisticated electronics cannot compensate for erroneous designs. As stated above, each process unit requires a specially designed sampling system. However, most sampling systems have a series of common features and specifications, namely:

(a) The sampling system should operate in a reliable way and require minimum maintenance for weeks or even months. This desirable feature is also extended to the entire process analyser. One such system requiring daily maintenance would be uneconomic.

(b) The sampling system should be fully automated, including sample collection, pretreatment and introduction into the analyser, stages which are often carried out manually in the laboratory. All this demands an appropriate initial design as there is no subjective judgement or human intervention in the process, unlike in laboratory work.

(c) The time taken to transport and condition the sample should be kept to a minimum. A very accurate and precise, although slow, analyser can hardly be of much use. The analyser system should be faster than the potential changes taking place during the process so that the technician in charge may act effectively on the operating conditions.

(d) The constituent materials of the sampling system should be carefully chosen so as to avoid contamination from the sample and damage to the whole system. The material used for each part of the system should be suited to the experimental conditions that it is meant to withstand. Stainless steel and special alloys such as Hastelloy C are typical construction materials here, while PTFE, chemical elastomers and stainless steel are commonly used as sealing materials —the use of materials such as copper, brass or carbon steel should be avoided because of their poor chemical consistency.

Every sampling system has two distinct parts: the sample conditioning system and the system for sample collection, transport and removal.

The sample conditioning system functions to change the nature of the sample extracted from the process line to adapt it to the analyser requirements. This is equivalent to the "sample pretreatment" carried out in the laboratory and involves the following steps:

(a) *Filtration of the sample.* Samples from an industrial process usually contain particulates such as boiler scales, tars from organic distillation columns and reactors, catalyst particles from fluidized-bed reactors and salt crystals. Their presence is undesirable from the point of view of maintenance and measurement reliability, and can give rise to background noise in photometers and short-circuits in electroanalytical instruments. These particulates are generally eliminated by means of filters of increasing mesh size located close to the sampling point. As with most of the components of the sampling system, it is usual to use several parallel sets and keep one working while the others are being maintained.

(b) *Elimination of the corrosiveness* or aggressiveness of the sample whenever possible, either chemically (acidity, alkalinity, inappropriate solvents) or physically (temperature, pressure).

(c) *Homogenization of the sample* so that it enters the analyser as a single phase, either the same as or different from that carried along the process line. This stage may involve: (1) vaporization of the sample by using

suitable pressures and temperatures; (2) separation of gases dissolved in a liquid by means of strippers (the sample may be either of the two phases); (3) separation of undissolved water from samples such as liquid hydrocarbons.

(d) *Buffering.* The addition of buffer solutions to the samples is often required in order to obtain a suitable medium for the chemical reaction on which the analysis is based to take place. Obviously, the buffer should contain no species that alter the measurement of the substance of interest.

(e) *Interference removal.* This is carried out by chemical conversion or masking. This step is usually imposed by the complexity of the samples extracted from process lines or waste effluents, which contain species disturbing the analysis or the analyser sensor.

(f) *Control of the sample pressure and temperature.* As a rule, the temperature and pressure of the freshly extracted sample are inadequate for introduction into the analyser and may alter the analysis or cause damage to the instrument; hence the need to control both variables, sometimes in a very precise manner. The sample pressure can be controlled with needle valves reducing the input pressure, followed by pressure regulators, which reduce the pressure to a given value irrespective of the input pressure —each pressure regulator must be followed by a pressure gauge. Keeping the sample at a constant temperature is generally more important than knowing its exact temperature. This can be accomplished by heating with electric heating coils or steam tracing —the production of steam and its use for this purpose are common in process plants where electricity is dangerous to use— or by cooling with cooling tower water or refrigerated brine in jacketed heat exchangers, common in process plants. However, there are cases where the temperature must be strictly controlled (e.g. to keep condensable liquids as vapour or volatile liquids as liquids). The use of heat conductors or isolators in each instance contributes in a passive way to the control of temperature.

(g) *Control of the flow-rate and size* of the sample entering the analyser. The control of the sample flow-rate to process analysers is as important as the control of its pressure, although easier to regulate on account of the simplicity of the instrument. Rotameters consist basically of a needle valve followed by a float in an upright transparent tube calibrated according to the fluid density. A pump is the best alternative whenever the pressure of a flowing stream is to be raised to a given value or a preset flow-rate is to be accurately fixed.

(h) *Partial or complete removal of water* from the sample, depending on the analyser requirements. The water content in a process line can vary between a few parts per million to the occurrence of an actual aqueous phase in non-polar currents. The sample moisture can interfere with the analysis by masking

or modifying some adsorption bands in infrared spectroscopy or photometry, or exhaust a chromatographic column prematurely. It can also foster corrosion of wet parts through the absorption of gaseous acids. The sample moisture can be removed by use of desiccating substances if the water content is low, or with coalescers if it is so high as to form a true second phase.

(i) *Sample dilution*, if required.

On the other hand, the system for sample collection, transport and removal is intended to extract the sample from the process line, transport it along the conditioning system to the analyser and evacuating back to the process line or to a waste container. All these operations require human intervention when carried out in the laboratory. The selection of a suitable design relies on several premises, namely:

(a) The sampling point should be appropriately chosen. It should never be the bottom of a pipe, where the settled matter may cause damage to the sampling system or the analyser, which would therefore require frequent maintenance. Neither should it be close to zones of high turbulence, high particulate levels, mixed liquid–vapour phases, pollutants or high temperatures or pressures. Under laminar flow conditions, the centre of the tubing is the point where the fluid circulates at the greatest velocity and hence the ideal sampling point.

(b) The extracted sample should be representative of the process stream, leaving aside undissolved water, suspended matter and other undesirable materials commonly present. Even the best designed analyser is bound to fail if it is not fed with a sample representative of the process to be controlled. This is achieved by: (1) choosing a sampling point not subject to turbulence and where only one phase is present; (2) taking the sample from the centre of the pipe in order not to include gases from the top or sediments from the bottom; (3) taking various samples at different points along the process line and, if necessary, mix the collected samples according to a known distribution. All these conditions can be fulfilled by a properly designed sampling probe.

(c) The sample composition should not change during its transport. Any vaporization or condensation during the transport should be avoided by controlling the sample temperature and pressure, as should the use of constituent materials fostering selective adsorption on their surface or degradation of key components of the sample. Rapid flows, short transport lines, wide diameters and inert materials contribute to minimizing these undesirable phenomena.

(d) Ideally, the sampling should involve various streams not subject to cross-contamination between one another, the use of standards for calibration of the instrument or "zeroing" —the use of blanks. Cross-contamination between streams can be prevented by using block and bleed stream-switching sys-

tems, *viz.* sets of valves allowing the introduction of a given stream into the analyser as required. The need to control a given number of sampling points without continuous monitoring or the use of several analysers (e.g. the determination of the oxygen profile in a furnace) can be met by performing automatic sequential samplings by means of systems with several modes or the complex systems developed by various manufacturers.

(e) The analysed sample and its non-analysed excess can be returned to the process line from which they were extracted or alternatively be expelled to a waste zone. However, samples containing noxious or hazardous species cannot be released without pretreatment if the laws on safety and environmental protection are to be complied with. On the other hand, some samples are best recovered for economic reasons.

### 16.4.2 Protection of the analyser equipment

Analyser systems are expensive and should therefore be adequately protected from their environment and the process streams that they control. Every analyser is liable to malfunction upon contact with rain, snow, ice, wind, sand, dust and so forth. After some time, alternate hot-cold or humid-dry periods cause expansions and compressions that results in erosion and corrosion of the analyser. On the other hand, industrial environments are particularly severe as the rain and atmospheric humidity react with traces of hydrocarbons, sulphurized products and nitrogen oxides to form acids which accelerate corrosion. All these reasons recommend protecting the analyser to an extent depending on the potential hazards of the area where the analyser is based, as well as on its robustness.

The analyser protection should comply with safety regulations, especially strict for hazardous areas, which involve protection of the workers, emergency actions, identification and handling of radioactive, toxic or inflammable products, protection against fire, control of the industrial environment, etc.

On-line analysers are usually placed in purpose-built houses [7]. These provide a suitable environment for the analyser, operators and maintenance personnel alike, a conditioned atmosphere ensuring the stability of the analyser, and the ideal way to link and allocate additional supplies such as electricity, air, water, vacuum, etc.

Large houses are sometimes built with bricks or of concrete. The average area of a typical house is 20x40 ft. A recent innovation is the installation of prefabricated steel panels with integral isolation similar to that of portable steel buildings, greatly accepted among builders. A major advantage of these houses is that the analysers can be installed long after the plant has been started with much less effort.

Small houses (6x8 ft) are often built with 2-in thick walls filled with foam inserted between reinforced plastic panels —similarly to modern refrigerators. The plastic material used should be non-flammable and non-toxic if it is to be exposed to fire. In addition, the construction should withstand winds of up to 200 km/h.

Houses located in hazardous areas should comply with the electrical regulations in force. In some cases, the whole house must be pressurized by using a source of fresh air in non-hazardous areas and an air-sensitive switch to automatically turn off the electrical power if the aeration system should fail. Even if the air is efficiently renewed, explosion-proof switches should be used for all electrical apparatus.

Fume detectors should be employed to detect leaks from the sampling system, especially when the fumes are toxic or flammable.

Another advantage of placing the analyser house at a strategic point is that the lines of the sampling system can be kept short and ancillary elements such as taps or stopcocks can be mounted outside the house —their right place. In some cases, the analyser house can be partitioned into two or three compartments, which can house the sampling system and the electronics (i.e. the analyser proper) and provide a space for repairs (Fig. 16.1).
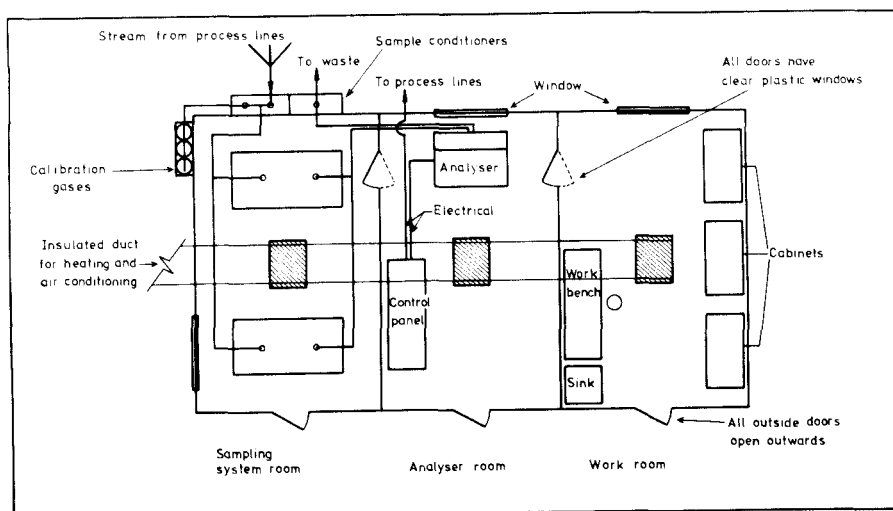


**Fig. 16.1** Typical analyser house.

The sampling system compartment should be isolated from the others by means of sealed conduits and fume barriers as it is the most likely origin of leaks. It should contain alarms warning of the presence of fumes or failures in the aeration system and hence of the safety inside the analyser house for the technicians devoted to the analyser operation and maintenance.

Each compartment should have two doors opening to both sides except for the external doors, which should open outwards and only from the inside. All doors should ideally be fitted with plastic or glass windows —the larger they are, the easier is the observation of the equipment. Detachable panels are of great help in cases of emergency. Figure 16.1 shows the scheme of a typical analyser house.

### 16.5 SCOPE OF APPLICATION

There are as many fields of application of process analysers as there are kinds of industrial plants. Their purposes vary with the plant in question: in oil refineries and nutrition industries they are intended to control product purity, either to meet scheduled specifications or so as not to cause detriment to the consumer's health. In other instances (e.g. power plants), they are meant to avoid corrosion of the materials making up the production plant, while in most cases —particularly in chemical industries, paper mills or blast furnaces— they are intended to control gas and liquid wastes to comply with social regulations.

Process analysers use a variety of detection techniques such as photometry, potentiometry, conductimetry and infrared spectrometry. However, according to Villalobos [8], gas chromatographs are by far the commonest detectors in this context (24% of all analysers currently in use), followed at a great distance by oxygen analysers and pH-meters. This is the result of the search for techniques with a high separation power (gas chromatography) or that are almost specific (oxygen analysis, pH measurements), avoiding the action of the potential interferents that commonly occur in the samples usually analysed.

The variety of analytes monitored by process analysers can be determined on the basis of their physical or chemical properties.

(a) *Physical properties* such as those fluid properties formerly used to control processes and currently used to condition the sample extracted from the process line: pressure, temperature, flow-rate. The physical properties most commonly controlled in this regard are conductivity, density, vapour pressure, refractive index, etc., which are properties of the fluid as a whole. Their changes in a process stream are caused by a single species which can thus be controlled indirectly.

(b) *Chemical properties.* Industrial process lines carry a variety of chemical species; both organic substances (hydrocarbons, carbohydrates, aminoacids, phenols, nitrosamines) and inorganic ions ($Cl^-$, $Na^+$, $H^+$) or molecules ($CO_2$, $O_2$, $H_2$). In all instances, the sample can be solid, liquid or gaseous. Because of the above-mentioned diversity, a comprehensive description of the applications of process analysers is beyond the scope of this chapter, which deals only with a number of representative examples.

*Oil refineries* involve a large number of different processes that require exhaustive control to meet product specifications imposed by the market. Below are discussed some aspects of the treatment of crude oils, for which the use of process analysers is mandatory [9].

Oil plants handle saline water, known as "formation water" and containing about 20% of salts, together with the product. This is an undesirable phenomenon which compels diversion of the well jet from the main stream to be refined. As a rule, 5-10 wells feed each gas-oil separation unit, intended to remove gas from the oil in three or four stages. It would be uneconomic to place one analyser in each well stream, so that analysers are placed only in the unloading line of each gas-oil separation unit. The analysers typically used here are salt-in-crude analysers and densitometers for low and high concentrations, respectively, of water in the oil.

In refining the oil it is necessary to stabilize the crude by removing the liquefied petroleum gases (LPG), generally containing substantial amounts of $H_2S$ and other sulphurized compounds that must be eliminated to protect the LPG plant and produce propane and butane suitable for sale. A Reid vapour-pressure analyser monitors whether the crude has been properly stabilized.

The hydrocarbon desulphurization process illustrates two different purposes of these analysers: process control and control of wastes dumped into the atmosphere. The desulphurization process involves two stages. In the first, sulphur is removed from the hydrocarbons by catalytic hydrogenation (Fig. 16.2):

$$R-SH + H_2 \rightarrow H_2S + RH$$
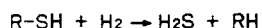
Then the $H_2S$ is removed from the hydrocarbon by selective absorption with triethanolamine. The feeding of hydrocarbons to the desulphurization unit is controlled automatically by means of an analyser according to the unit yield. For economic and environmental monitoring reasons, the amine stream is passed through a sulphur recovery plant based on the Claus process. This involves oxidation of hydrogen sulphide to sulphur to a extent of 90% and storage of the sulpur. The high yield is achieved in two steps controlled by an $H_2S$ analyser (a gas chromatograph with a flame photometric detector) ensuring the produc-

tion of an adequate H2S/SO2 mixture. The hydrocarbon concentration entering the Claus plant shoud be kept to a minimum in order to avoid deposition of carbon on the catalyst surface —this is controlled by a gas chromatograph. The gas emerging from the Claus plant usually contains too much H2S (roughly 10%) to be released to the atmosphere, so that it is passed through oxidation furnaces and absorbers to reduce the H2S concentration to about 10 ppm (mL/m$^3$).
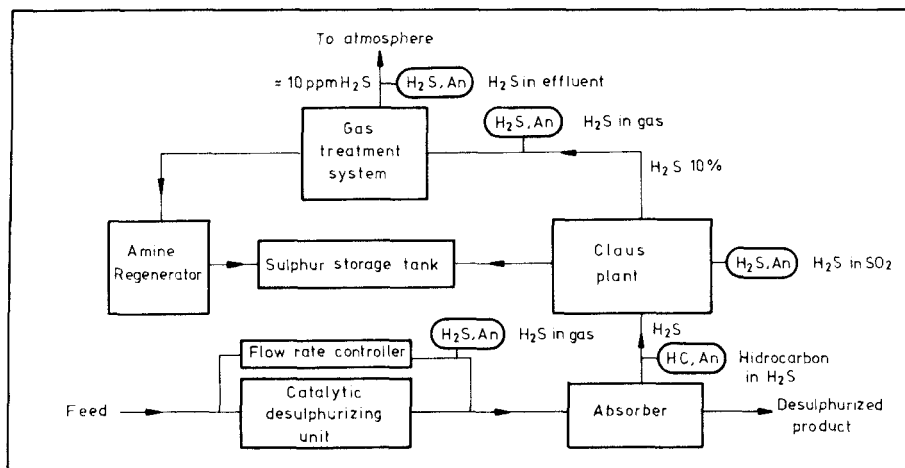


**Fig. 16.2** Simplified process of desulphurization and recovery of sulphur in a refinery. (H2S, An) and (HC, An) denote the H2S and hydrocarbon analysers, respectively.

Hydrogen analysers are frequently employed in this type of industry as a result of the usual hydrogenation of petroleum derivatives (naphthas, acetylenes). These instruments are based on the measurement of the gas density or thermal conductivity. They are typically employed in the reforming of naphthas. These compounds are hydrogenated prior to mixing with other petroleum derivatives in the production of gasolines. In Fig. 16.3 is shown a typical assembly for reforming of naphthas including an automatic system for control of the temperature of the naphtha entering the reformer based on the measurement of the octane number of the reformed product. Figure 16.3 also shows a

simplified system for regeneration of the catalyst used in the hydrogenation. This type of catalyst must be regenerated when the hydrogen concentration in the recycled gas falls below 55% by passing a hot air stream over the catalyst. This oxidizes the deposited carbon to $CO_2$ and is controlled by an oxygen analyser located at the hot air outlet.
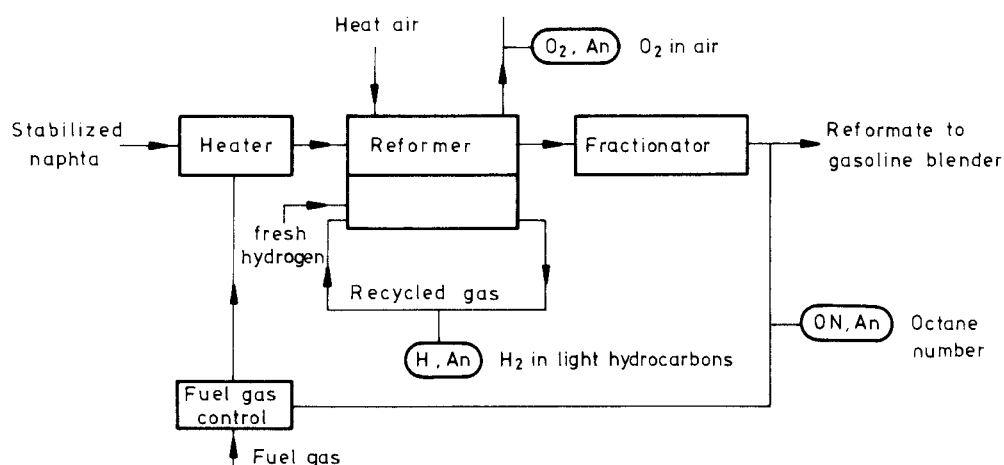


**Fig. 16.3** Reformer unit control based on reformate product octane rating setting the temperature of the reactor feed, and catalyst regeneration system. ($O_2$, An), (H, An) and (O.N., An) are the oxygen, hydrogen and octane number analysers, respectively.

As stated above, *gas chromatographs* used in process control are the analysers most commonly used in the industrial field. This is particularly true of oil refineries and petrochemical industries as a result of the versatility, selectivity and suitability with respect to the products to be analysed, *i.e.* gases or volatile liquids. These instruments have been used for a variety of applications: control of ambient air in industry (determination of vinyl chloride), of reagent purity (determination of traces of water in xylenes) and of processes (determination of the products obtained in the hydrogenation of acetylenes). These instruments are widely used in the fractionation of LPGs (Fig. 16.4) in controlling fractionators to ensure their optimum and economic operation with acceptable impurity levels.

Process analysers less commonly used are employed in lube oil and wax treatment plants (Fig. 16.5). In a first stage differential refractive index

analysers are used to control the viscosity in order to ensure the efficient separation of aromatic oils from the feed. In a second stage, the feed is cooled until the wax crystallizes and can be separated from the oil by fil- tering. The plant operation is controlled by a pour-point analyser. The oils are then treated for acceptable coloration and the wax is purified, thereby obtaining refined products suitable for sale. Colour and melting point ana- lysers are employed for these purposes.
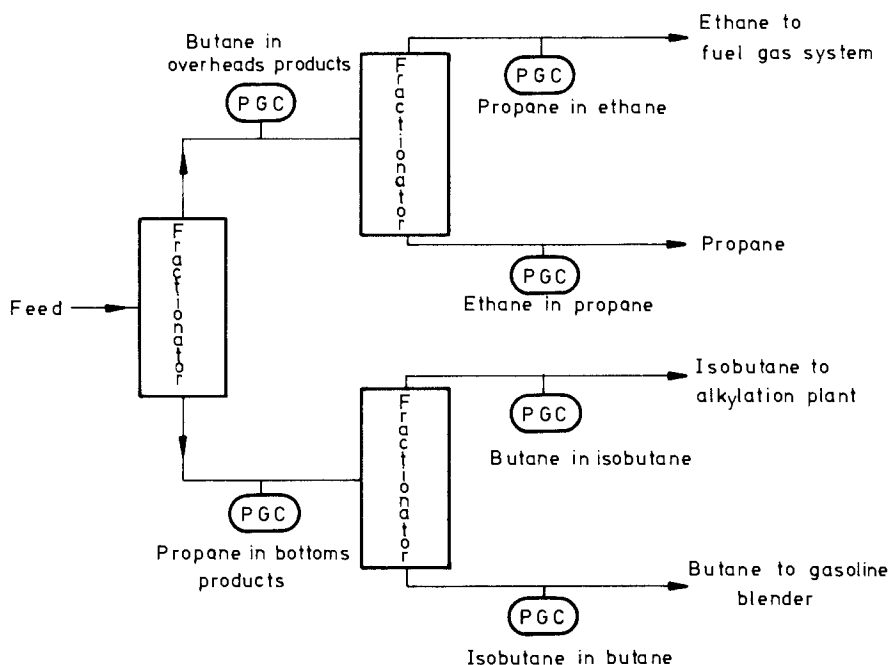


**Fig. 16.4** Location of petrol gas chromatographs in a typical LPG frac- tionation plant for light hydrocarbons.

The power consumption in the *iron and steel industries* amounts to 20% of the total energy used by the USA, most of which is obtained from coal [10]. The conversion of coal to coke can be exploited in three ways: (a) coke is a source of heat and a blast furnace fuel for the production of pig iron; (b) one-third of the coke oven gas (COG) is used as fuel for underfiring coke fur- naces; (c) the other two-thirds of the COG is used for reheating of furnaces in steel production plants. These reasons make necessary the analysis of COG and the fumes from blast furnaces and steel production plants —mainly $H_2$, $CH_4$, $CO$, $CO_2$ and $O_2$). Prior to analysis, the samples must be conditioned (Fig. 16.6) by removing tar, water and light oils. Hydrogen is normally measured by
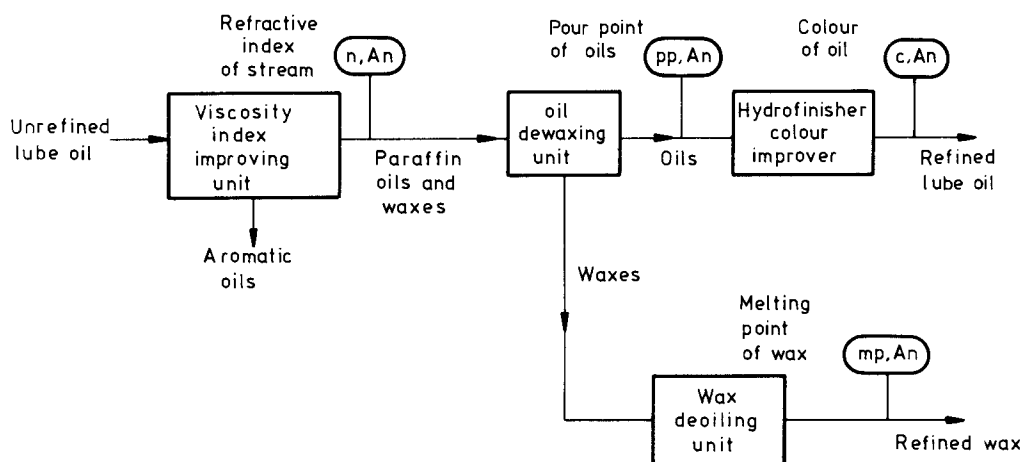
**Fig. 16.5** Block diagram of a lube oil and wax treatment plant. (n, An), (pp, An), (C, An) and (mp, An) are the refractive index, pour point, colour and melting point analysers, respectively.

a thermal conductivity detector and methane is determined by gas chromatography. Carbon monoxide and carbon dioxide are determined by IR spectroscopy, while oxygen is sensed by a paramagnetic analyser. The control of the oxygen content of COG is important insofar as the inflow of air to the furnace increases the volume of gas to be processed, causes the combustion of carbon rather than the formation of coke and, especially at concentrations above 5%, may cause the formation of explosive mixtures (55-60%) with the hydrogen present in the COG.

*Oxygen analysers* are commonly used in most industrial processes, where oxygen occurs as a gas or dissolved in a liquid phase. Gas analysers are used with a variety of detectors: $ZrO_2$ and Tl cells, thermomagnetic and paramagnetic. Analysers for oxygen in liquids can be galvanic or polarographic. Those used to determine oxygen in gases are employed for a variety of purposes: (a) in systems for the control of combustion air with the aim of optimizing the combustion efficiency and hence decreasing fuel consumption and the production of unwanted gases; (b) in controlling stack gas; (c) in controlling air leak-
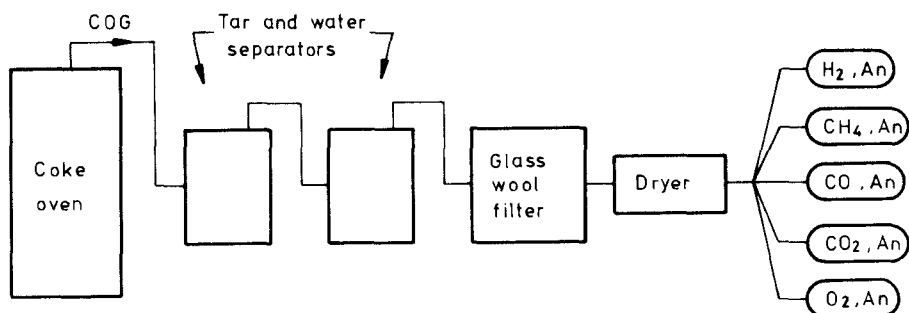
**Fig. 16.6** System for conditioning of coke oven gas. On the left are depicted the hydrogen, methane, carbon monoxide, carbon dioxide and oxygen analysers.

age; (d) for control of oxygen in non-ventilated industrial areas; (e) for control of inert gas purges to prevent oxygen from degrading or contaminating the contents of storage tanks; (f) in controlling catalytic regeneration systems in petrochemical industries. Dissolved oxygen analysers have completely different uses, namely: (a) in controlling pollution in rivers, lakes, streams and industrial plant effluents; (b) in controlling and improving the efficiency of aeration systems; (c) for the control of water treatment plants to ensure the oxygen levels required for adequate bacterial growth; (d) for the control of beverage and food processing plants —oxygen can detract from the quality of foods and drinks—; (e) in controlling fermentation processes frequently involving certain amounts of oxygen.

In addition to some components such as tar, ammonia, naphthalene and aromatics, which are removed during the gas scrubbing, coke oven gas contains a significant concentrations of HCN, $H_2S$ and other sulphurized compounds. As COG is employed as a fuel in various production processes in the iron and steel industry, it must be purified as the gases released to the atmosphere would exceed the permissible levels. Hydrogen cyanide is eliminated by catalytic degradation and $H_2S$ is removed by treatment with an aqueous solution of monoethanolamine or ammonia, or by the Thylox process. Prior to their release, the fumes are measured for their HCN and $H_2S$ contents by means of a gas chromatograph with a thermal conductivity detector.

The use of process analysers in the *food industries* is not so common,

probably because of their traditionalism and attachment to conventional systems. Thus, in breweries, processes are chiefly controlled by measuring physical properties such as the temperature, flow-rate, liquid level or pressure, while the quality of the end product is controlled almost entirely in the laboratory. However, there are many points along the process where analysers are used (Fig. 16.7). Beer production involves three essential stages: wort production, fermentation and filtering, and storage. During the first stage, malt starches and proteins and the adjuncts are hydrolysed to simpler substances such as sugars and amino-acids. The water required for wort production must be very pure, so it is treated with active carbon or ozonized to make it odourless and microbiologically sterile —the last treatment involves the use of an ozone analyser to ensure that the ozone concentration is not so low as to detract from its biocidal power, or so high as to corrode the piping. During wort production, some brewers determine the oxygen content, although this phase is generally controlled by measuring the temperature and density —known as "original gravity". The fermentation process basically involves the anaerobic degradation of maltose to ethanol and $CO_2$. This process must be carefully controlled so that, in addition to thermometers and densitometers, instruments for measurement of the yeast concentration (electronic particle counters), the oxygen content, which should be minimum as the process is meant to be as anaerobic as possible, and the $CO_2$ concentration, which is useful not only to control the fermentation but also to indicate the purity of the gas produced in this phase, which is stored, are required. Finally, time should be allowed for the deposition of suspended solids, after which the product is filtered under nephelometric control to ensure a proper appearance. Prior to bottling, an accurately measured amount of the stored $CO_2$ is added to improve the odour, flavour and frothing of the product.

*Carbon dioxide analysers*, based on heat capacity, electrical conductivity or partial pressure measurements, but especially on IR light absorption, are therefore used at various points in industrial processes. Carbon monoxide and dioxide are the species most commonly analysed for by the last of the above-mentioned detection techniques, which is also employed in a variety of processes [6], namely: (a) control of the hydrogenation of plant oils in order to avoid production of unwanted *trans* isomers; (b) measurement of sugars and $CO_2$ in soft drinks; (c) measurement of moisture; (d) determination of $CO_2$ in industrial environments; (e) determination of isocyanates in the production of polyurethane; (f) determination of methane in argon from nuclear plants; (g) control of the efficiency of solvent clean-up and recovery systems.

*Power plants* use various types of process analysers. One of their purposes is the control of boiler scales. These pose two major problems: (a) high
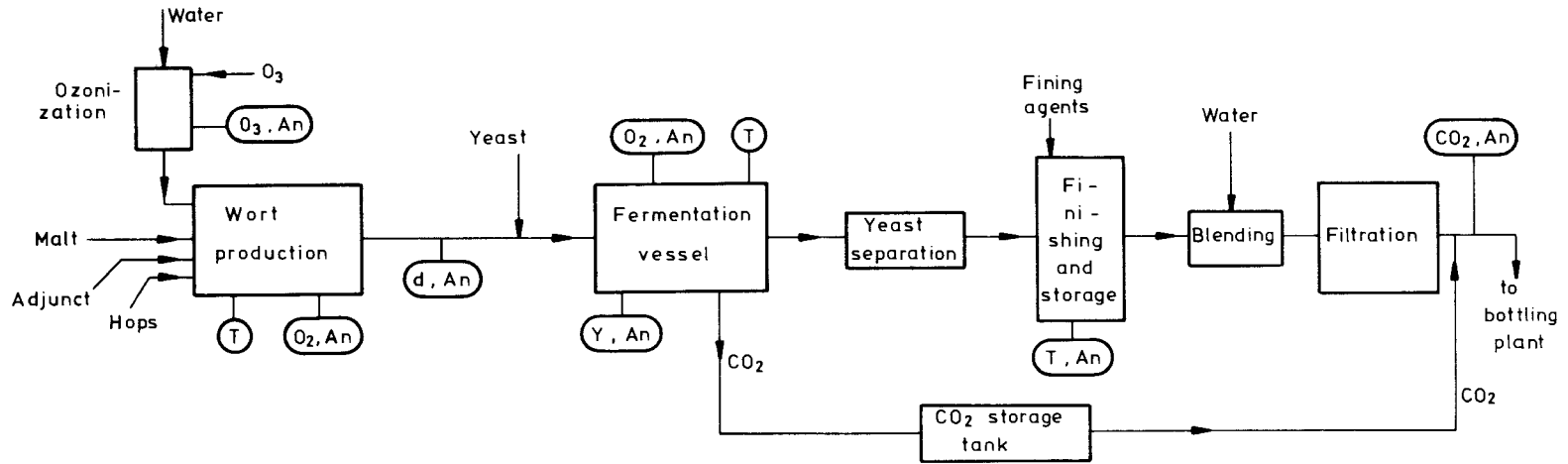
**Fig. 16.7** Block diagram showing the different steps involved in brewing. ($O_3$, An), ($O_2$, An), (d, An), (Y, An), (t, An) and ($CO_2$, An) denote the ozone, oxygen, density, yeast, turbidity and carbon dioxide analysers, respectively. (T) is a thermometer.

chloride concentrations attack stainless-steel parts of the system, thereby fostering their corrosion; (b) the presence of scales lowers the heating efficiency of the water boiler. As an enormous amount of water is evaporated from the boiler, the solids concentration must be very low —of the order of a few µg/L—, so that very sensitive analysers are required. The steam production system is usually furnished with units to help reduce solid deposition and corrosion of the boiler and condensers (Fig. 16.8), either by means of chemical additives such as $Na_2HPO_4$, which inhibits the precipitation of calcium and magnesium and coats and passivates the tubing surface, and $Na_2SO_3$, which removes excess oxygen from the boiler water and hence prevents corrosion by piercing of the boiler tubes, or by use of condensate polishing systems or mixed-bed demineralization systems, which decrease the amount of condensed solids, without laborious elimination. The analysers most commonly used in this field are conductimeters and sodium-selective electrodes. In some cases, when the analyser detects a salt concentration above a preset value, the unit is halted automatically —this obviously calls for conductimeters, which are more reliable than ISEs. Conductimeters located downstream of the condenser allow the system to be stopped as a valve is opened to expel contaminants —which are therefore prevented from reaching the water tank— and an alarm is triggered for the failure to be remedied.
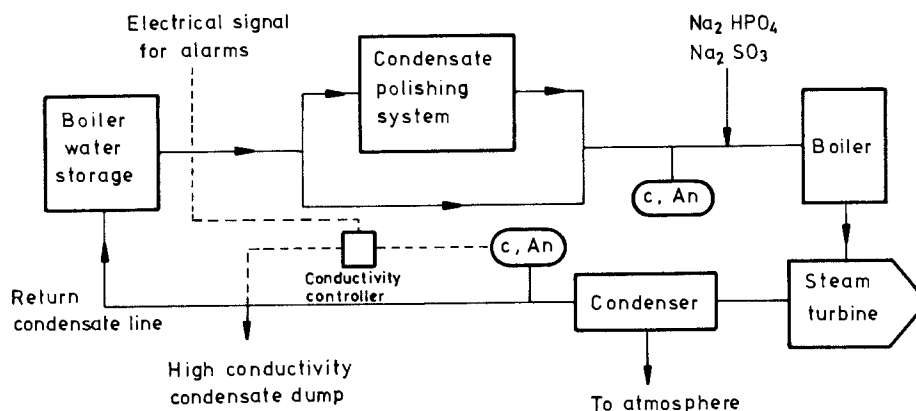


**Fig. 16.8** Block diagram of a steam condensate return or dump based on conductivity measurements (c, An) in a power plant.

*Conductimeters* used for process analysis are as simple as those using a Wheatstone bridge as a detector or as sophisticated as electrodeless conductivity systems. In addition to those described above, they are employed for a

host of purposes, namely: (a) for measuring the conductivity of the fluids from cooling towers with a view to controlling evaporation losses and unnecessarily increased amounts of dissolved solids; (b) for the detection of dripping corrosive materials through process heat exchangers; (c) for controlling waste water, filtration of saline water to springs, ponds or wells; (d) for controlling the efficiency of the production of distilled or de-ionized water; (e) for the determination of the concentration of acids, bases or salts in various chemical processes in brine or caustic degreasing baths, anodizing solutions, etc.

Other functions served by process analysers include the analysis of gases produced at different points in *nuclear reactors*. Among such gases are hydrogen, methane and other hydrocarbons formed as lube oil drips into the $CO_2$ heat exchanger and is cracked at the high temperatures reached within the reactor. The control of contamination by $H_2$ and $CO_2$ of the condenser when using graphite moderators is also important. Finally, the vapour pressure at the boiler outlet is much higher than that of $CO_2$ in the condenser, so that some vapour may find its way into it. The use of a gas chromatograph in the first two cases and a moisture meter in the last allows the detection of the corresponding anomaly.

As a rule, water traces are difficult to measure because of the very nature of water and of its high content in the atmosphere. In addition, water tends to be adsorbed on almost any type of surface and to build up in all crevices of the system. Moisture has an adverse effect in many respects: it can degrade stored food, lower the quality of end products, damage electronic components, decrease the efficiency of catalysts and, in many cases, considerably reduce the lifetime expectations of many elements or process plants. There are several types of *hygrometers*: electrolytic, suitable for measuring low moisture contents in corrosive gases; those based on impedance or steam pressure measurements, usable as in-line analysers in non-corrosive gas streams and non-polar liquids; and those based on IR light absorption; those using hygroscopically coated quartz crystals and oscillating circuits. They have a variety of applications: (a) measurement of moisture in anhydrous gases with the aim of keeping it to a minimum in order to avoid corrosion of piping and process containers; (b) control of humidity in rooms housing electronic material; (c) control of moisture in tempering furnaces and filling or isolation gases; (d) control of moisture in catalytic reforming operations to avoid catalyst passivation; (e) control of moisture in hydrocarbon streams obtained by cryogenic fractionation or distillation to avoid the formation of ice or liquid water that might block the conduits; (f) control of moisture in effluent gases from dryers and condensers in order to determine the process

efficiency; (g) control of moisture in gases with a view to augmenting the efficiency of combustion of hydrocarbons —water is one of the products generated in this process.

A recent application of graphite-furnace atomic absorption spectrometry in nuclear plants is the purity control of primary-side water, necessary because some species occurring in water, such as aluminium, calcium, magnesium and silica, can contribute to the formation of undesirable crude deposits on the nuclear fuel cladding and should therefore be reduced to very low levels (a few µg/L) in the primary water.

Process analysers are widely applied to the *control of pollutants*. Some of the applications (e.g. the removal of HCN and H₂S from coke oven gas and the control of CO₂ in breweries) were commented on above, and a few others are discussed below.

Sunlight causes the reaction of atmospheric hydrocarbons with nitrogen oxides to form photochemical smog; in addition, the noxious effects of gases such as CO, CO₂, NO and NO₂ are well known. One of the principal sources of emission of these gases to the atmosphere is internal combustion engines, so they must be tested to ensure that they do not emit more than the maximum allowable concentrations of various pollutants. The motor vehicle is tested on a roller brake with a programmed cycle of idling, acceleration, running, gear changing and deceleration. Figure 16.9 shows the analyser for gases
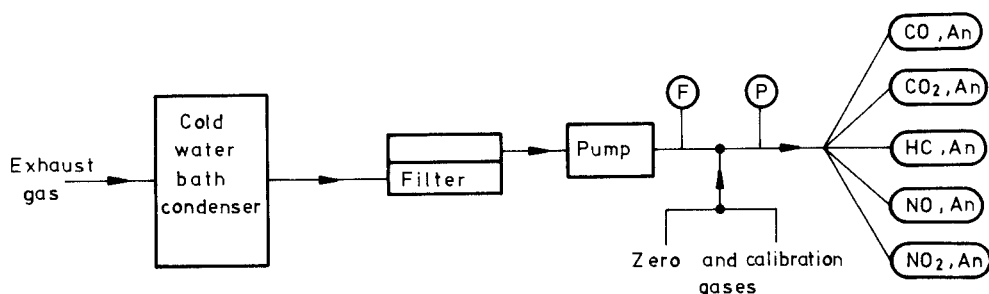


**Fig. 16.9** Simplified diagram of a system for analysis for exhaust. (F) and (P) denote flow-meter and pressure gauge, respectively. (CO, An), (CO₂, An), (HC, An), (NO, An) and (NO₂, An) are analysers for CO, CO₂, hydrocarbons, NO and NO₂.

emitted by motor vehicles. The gas pretreatment includes condensation of the

water produced in the combustion, filtering and pressure control. The analyser possesses a system for zero-setting and calibration of each detector. Normally, NO, CO and $CO_2$ are measured by IR spectrometry, as are hydrocarbons, which can also be determined by gas chromatography. Nitrogen dioxide is determined by UV spectrometry.

The pulp and paper industries produces three types of pollution: foetid gases and particulates, polluted water used in various processes and noise. The chief gas pollutants produced in brewing are reduced sulphurized compounds [$H_2S$, $CH_3SH$, $(CH_3)_2S$, $(CH_3)_2S_2$] commonly known as 'total reduced sulphur' (TRS), and sulphur oxides ($SO_2$, $SO_3$), nitrogen oxides (NO, $NO_2$) and other organic compounds such as terpenes, hydrocarbons, alcohols and fenols released by wood pulp. The large amounts of pollutants produced necessitate the use of systems for their elimination and analysers to measure their concentrations in the gases dumped into the atmosphere, which must be kept within the limits established by legislation. The analysers typically used to control sulphurized compounds (gas chromatographs with flame photometric detectors), CO, $CO_2$, NO (IR analysers) and $NO_2$ (UV analysers), were commented on above, so that only the systems for collection and conditioning of TRSs are described here.
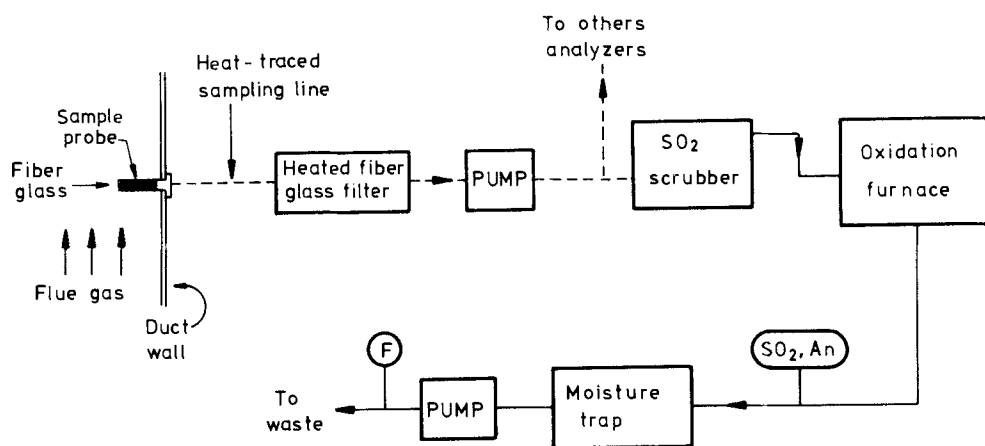


**Fig. 16.10** Sampling and conditioning system for analysis of total reduced sulphur (TRS) compounds. (F) and ($SO_2$, An) denote the flow-meter and the $SO_2$ analyser, respectively.

The system depicted in Fig. 16.10 consists of a sample probe with a glass fibre filter for the elimination of coarse particles from the gas stream, followed by another filter for retention of finer particles. This last filter is heated, as are all the sample transport lines in order to avoid steam

condensation, which might result in clogging. The $SO_2$ is removed by bubbling the sample through  potassium hydrogen phthalate, which also retains excess steam and particles that may remain in the gas and cools the gas to ambient temperature. Subsequently, the compounds in the TRS fraction are oxidized in a furnace as the potentially interfering terpenes and aromatic and olefinic compounds are eliminated by degradation to $CO_2$ and $H_2O$. The resulting $SO_2$ is determined by means of an appropriate detector.

The particulate concentration in stack fuel gases must be determined to comply with current legislation on environmental pollution, which places emphasis on the particle size. By "particulate matter" is understood any solid or liquid material emitted to the atmosphere, including dust, fumes, ash, soot, tar and droplets. These "analytes" can be monitored continuously by means of detectors based on the absorption of light or β-radiation or on the transfer of charge between particles.

*Water-treatment plants* still use process analysers fairly infrequently; however, the water chlorination process is an exception to this rule. Chlorination is the commonest water sterilization method. The speed of addition of chlorine at a given moment depends on the water demand, the pH and the amount of organic matter and bacteria present. On the other hand, the residual chlorine level in the sterilized water should be sufficiently high (0.2-0.3 μg/mL) to ensure that the water is effectively sterilized and that bacteria subsequently contaminating it are destroyed. However, excess residual chlorine is a waste and endows the water with unpleasant odour and taste. This compels to appropriate control of the treatment. Figure 16.11 depicts a typical water chlorination system in which the chlorinator adds chlorine to the water; the water is sterilized for about 30 min and then measured for its content of residual chlorine. If such a content is too low, more chlorine is added; if it is too high, the ammoniator dispenses the amount of ammonia required to counteract the excess chlorine by formation of chloramines, which are odourless and have less bactericidal power, although they are more persistent, than chlorine. In this case, a pH-meter is used to measure and set the pH of the water to a slightly acidic level where the bactericidal action of chlorine is the strongest.

The use of *pH-meters* is common because of the strong influence of pH on any kind of reaction [11]. Thus, they are used: (a) to measure the pH of heat exchanger liquids in order to detect possible stream leaks to the inside of the exchanger, as excessive acidity fosters corrosion of the system while excessive alkalinity favours scaling; (b) to measure the pH of plant effluents returned to the ecosystem as industrial waste treatment effluents and waste hold pond outlets, whose acceptable pH is usually between 6 and 9; (c) to

control electrolytic processes; (d) to neutralize electroplating wastes; (e) to control fermentation processes in pharmaceutical industries; (f) to perform studies on high-purity water; (g) to control the operational conditions in a variety of processes in the paper, brewing, soap and chemical manufacturing industries.
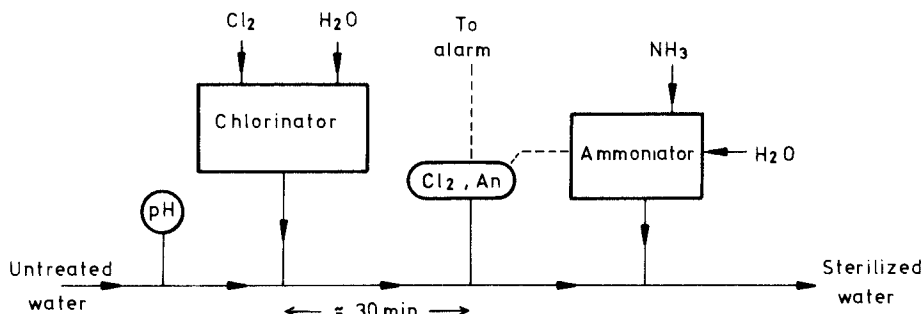


**Fig. 16.11** Conventional water chlorination system.

*Corrosion meters* are not used to determine the concentration of a given species or the value of a certain physical property in controlling a process, but to give a measure of the aggressiveness of the environment to equipment carrying out a given process. Corrosion is a continuous, natural process that can be slowed, although not stopped. In fact, it cost the process industry over 1 billion dollars per annum. Reducing the corrosion rate as far as possible minimizes this cost, increases the equipment's useful lifetime and saves energy, natural resources and maintenance. There are two chief types of corrosion meters: those measuring the instantaneous corrosion rate (ICR), also known as 'linear polarization resistance' (LPR), which are only used with polar or highly conductive liquids, and those measuring the electrical resistance (ER), employed with gases and non-polar liquids. The former are used in many circumstances, namely: (a) in oil field evaluations of inhibitors to control water, oxygen, $CO_2$ and brine, thereby allowing the determination of the efficiency of inhibitor formulae, their continuous or discrete dosing and the minimum level of inhibitor required; (b) in checking water injection systems to evaluate biocides, oxygen scavengers and filming inhibitors; (c) in measuring corrosion in process equipment and connecting pipe systems; (d) for

the determination of the corrosion resistance of metals and alloys in chemical manufacturing plants; (e) for studying the influence of pH, temperature, flow-rate and various pollutants on the corrosion speed; (f) for studying passivation film building and the anodic protection technique; (g) for the selection of materials for construction of industrial equipment; (h) for the optimization of de-aeration processes and the composition and temperature of the feed stream in order to minimize corrosion in brine evaporators; (i) in controlling the removal of acidic gases from natural gas production plants. Electrical resistance analysers are often used in oil refineries and chemical processing plants. They are also useful for the determination of the effect of polluted air on electronic equipment and in controlling air renovation systems in closed spaces.

### 16.6 MICROPROCESSORS IN PROCESS ANALYSERS. TRENDS

The incorporation of digital systems in process analysers is relatively recent owing to their former high cost; however, continuous technological breakthroughs have favourably influenced the optimization of these systems and contributed to their current affordability and presence in analysers.

Formerly, a computer was economic only if it was used to control several analysers —at least seven in the case of gas chromatographs. A central computer dealt sequentially with the programs of the analysers according to preset priority criteria (*time-sharing systems*). The usual case today is that a more or less powerful microprocessor controls a single instrument and its associated peripherals (*dedicated computer systems*). In large industrial facilities such as oil refineries, the microprocessors controlling the analysers should be interconnected, making up *computer networks*. In this way, if a computer should fail, only the plant that it controls rather than the whole system will be affected; on the other hand, through one of the microprocessors, the plant technician can obtain information from any of the computers in the remainder of the plants without the need to be physically present there, thereby saving time and improving the control of the plant as a whole.

The data obtained by the analyser are supplied to the technician through a terminal provided that four requirements are met: (a) the analyser should be suitably linked to the computer via an interface; (b) the analyser should transmit signals 'understood' by the interface; (c) the computer program should be capable of collecting the data sent by the analyser and storing them in bulk memory; (d) the computer should have a program for the retrieval, treatment and delivery of stored data.

Figure 16.12 shows a simplified scheme of a computer-controlled process

analyser. The key to the microprocessor is the central processing unit (CPU), which controls both the analysers and its peripherals. By means of a keyboard the analyser's operating conditions are controlled and modified via the analyser control and the interface. Likewise, the CPU receives data from the analyser through the interface and stores them if required. Then it processes the data and delivers results through a visual display (CRT), a printer or a continuous recorder. If the results are considered hazardous by the microprocessor, it triggers the pertinent alarm(s) and displays a warning on screen. Alternatively, a faulty analyser only communicates the computer's failures.
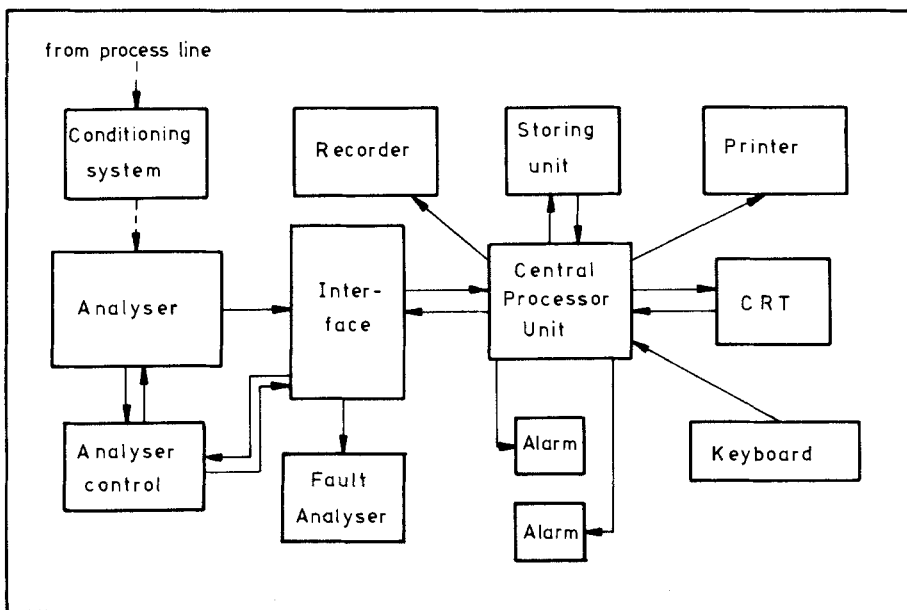


**Fig. 16.12** Block diagram of analyser controlled by a dedicated computer.

Microprocessors can serve a variety of functions in process analysers, namely; (a) improvement of data acquisition through rapid collection and storage for subsequent retrieval when needed; (b) data treatment according to the detection technique used —this ensures the faster and more convenient obtainment of more accurate results by avoiding human intervention; (c) result delivery as required by the plant technician (in digital or printed form, as a listing on a screen, on a continuous recorder permitting control of the signal evolution, etc.); (d) programming of the analyser operation —this allows the

elimination of the mechanical elements formerly used and enables the user to know and alter the operating conditions as and when required from the control room without the need to move to the analyser site; depending on the type of analyser, the system programming can be fairly simple (pH–meters) or complex (gas chromatographs); (e) automatic baseline correction to avoid excessive drift and decrease the background noise, and hence increase the precision; (f) calibration and recalibration of the instrument according to preprogrammed cycles which allow the accuracy of measurements to be maintained; (g) trouble-shooting by means of sensors, electronic units and other accessories —this, together with the improved alarm systems currently in use, considerably increases the control of the technician in charge of the plant; (h) interpretation of data and acting on the process line, which represents almost complete automation of the plant control.

A large variety of analysers such as ion chromatographs, mass spectrometers, digital titrators, calorimeters, specific ion analysers, gas and liquid chromatographs and octane number analysers are manufactured with built-in microprocessors.

Current developments in the field of process analysers seem to point to clear targets, namely: (a) increasingly larger memory storage capacity and speed with the advent of 32-bit microprocessors; (b) the development of a universal data highway that will facilitate the complete automation of industrial plants —robotization and artificial-intelligence analysers are longer-term objectives; (c) the increasing use of IR spectrometry and liquid chromatography in contrast to the prevailing gas chromatography; (d) the miniaturization of analyser elements, particularly sensors [12] and electronic components; (e) the increasing use of flow-injection analysis, a novel technique for the analysis of liquid samples characterized by its economy of sample, reagent and instrumentation, and its ready automation [13,14].

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